CHAPTER IV

RESULTS AND DISCUSSION

A. Extraction of Active Constituents from *Garcinia mangostana*

The crude extract of *Garcinia mangostana* was obtained by maceration of four kilograms of the dried fruit hull powder with ethyl acetate for extraction of non polar substances. The ethyl acetate extract was brownish viscous liquid, which was concentrated by using a rotary evaporator. Then the extract was crystallized into yellow crystal and ground into fine powder for further studies (Figure 28). The photomicrograph of yellow bright needle shaped crystalline of mangostin obtained is shown in Figure 29. The weight of final yield was 324.5 g, which was calculated as 7.47% yield.

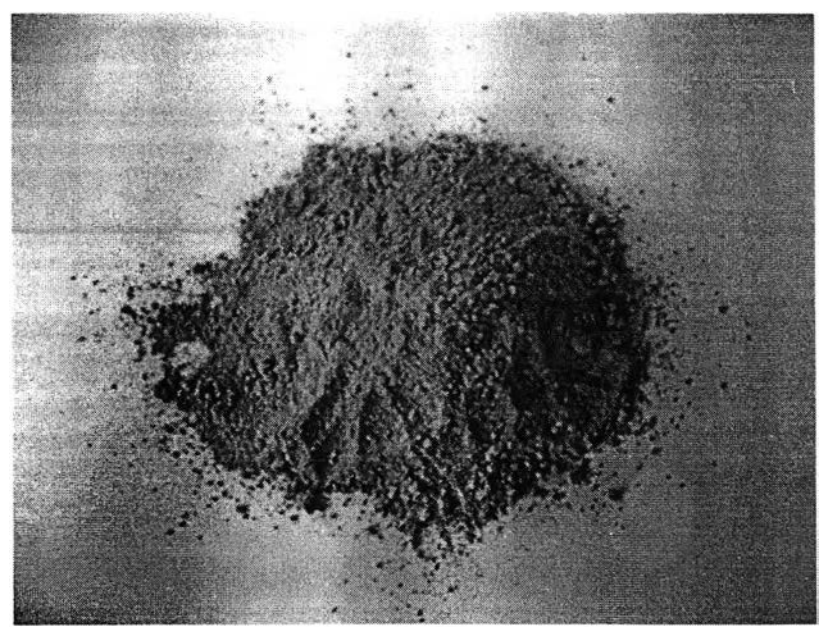
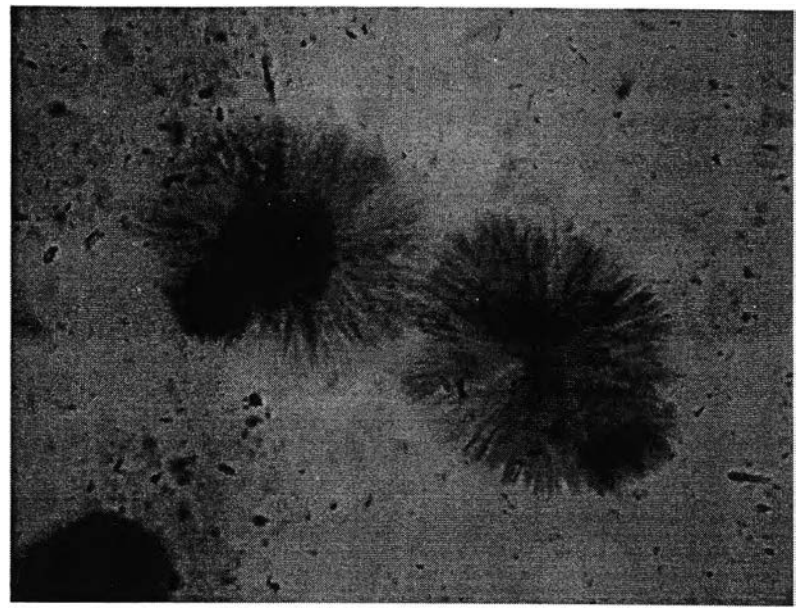
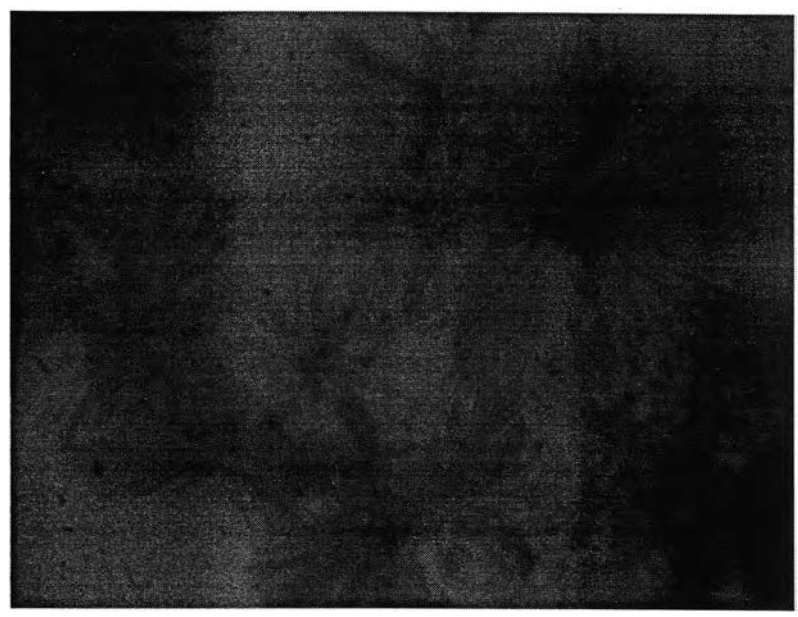


Figure 28 Photograph of fine powder of *Garcinia mangostana* extract



(a)



(b)

Figure 29 Photomicrographs of needle shaped crystalline of mangostin in extract (Magnification X 100)

B. Identification and Determination of Active Constituents from *Garcinia mangostana*

1. Differential scanning calorimetric (DSC) method

The information from differential scanning calorimetric (DSC) method can be used for compound identification or in an estimation of purity. The melting point of a substance is defined as the temperature at which the solid phase exists in equilibrium with its liquid phase (Brittain, 1995).

The accurately weighed amount of the extract was placed into an aluminum pan and the run was performed at the heating rate of 10 °C/min in the temperature ranging from –50 to 220 °C under nitrogen atmosphere. The experiment was performed in triplicate. The DSC thermogram of the extract showed an endothermic melting peak with a broad range of temperature at 165.04-166.80 °C (Figure 30), while the melting point of mangostin which was reported as 181.6-182.6 °C (Gopalakrishnan, Banumathi, and Suresh, 1997; Budavari, 2001). This result indicated that the extract might contain a mixture of other constituents such as 8-desoxygartanin (mp 155-156 °C), BR-xanthone (mp 180-182 °C), gartanin (mp 164-166 °C), or β-mangostin (mp 178-179 °C). The DSC thermogram of standard mangostin used in this study is provided in Appendix A, Figure A4 that showed the melting point at 184.48 °C

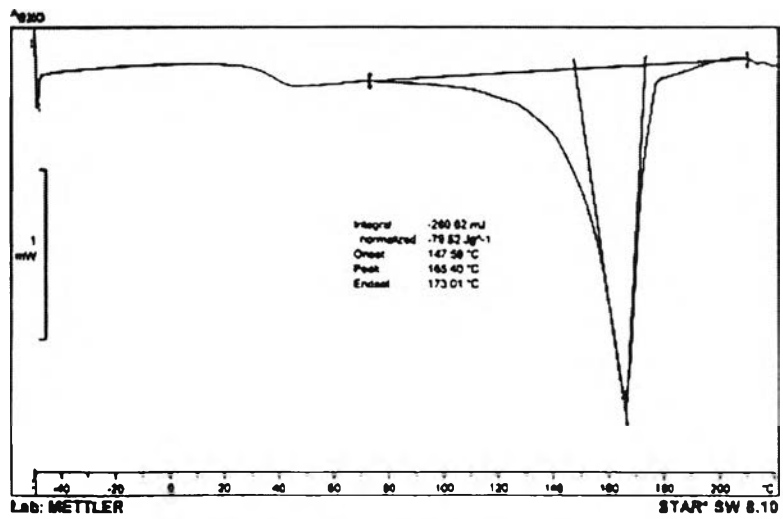


Figure 30 DSC thermogram of *Garcinia mangostana* extract (sample weight 3.27 mg)

2. Thin layer chromatographic (TLC) method

The isolated compound from dried fruit hull extract was identified by TLC. The chromatogram of the compound was compared to the standard mangostin as shown in Figure 31. In this chromatogram, the Rf value of the extract was equal to the standard mangostin at the value of 0.60. Based on this data, major composition of this extract was mangostin.

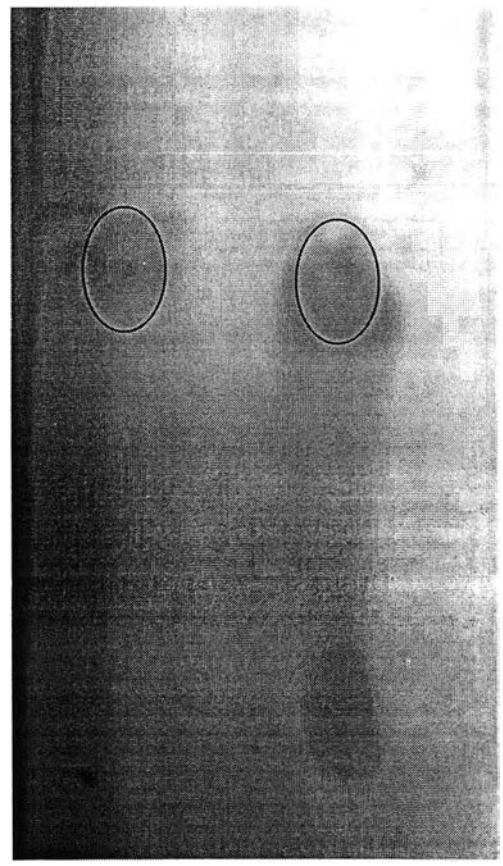


Figure 31 TLC chromatogram of (a) standard mangostin and (b) *Garcinia mangostana* extract.

3. High performance liquid chromatographic (HPLC) method

The developed HPLC system was applied to analyze the extract from dried fruit hulls of *Garcinia mangostana*. It was found that the extract had similar chromatogram to the standard mangostin. However, in the same concentration the extract gave the peak area ratio of 55.86 $\pm$ 0.22 % of the standard mangostin. This result is consistent with the information from DSC that the extract might contain a mixture of other compounds.

3.1 Validation of HPLC method

The validation of analytical method is the process by which it is established that the performance characteristics of the method meet the requirements for the intended analytical applications. The performance characteristics are expressed in term of analytical parameters. For HPLC assay validation, these include specificity, linearity, accuracy and precision.

3.1.1 Specificity

The specificity of an analytical method is its ability to measure the analyte accurately and with specificity in the presence of other components in the sample.

The internal standard technique was performed by determining the peak area ratio of mangostin to clotrimazole (internal standard) to give the complete separation, appropriate resolution and sharp peaks of all components. The methanol-water mixture of 87% by volume was used as the mobile phase. The typical chromatograms of blank solution, internal standard solution, mangostin standard solution and *Garcinia mangostana* extract solution are shown in Figure 32-37.

The retention times of internal standard solution, mangostin standard solution and *Garcinia mangostana* extract solution were around 5.073, 8.844, 8.872 min, respectively. In addition, there was no interference from other components in the chromatogram.

3.1.2 Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The linearity is usually expressed in terms of the variance around the slope of the regression line calculated according to an established mathematical ralationship from test results obtained by the analysis of samples with varying concentrations of analyte. The calibration curve data of mangostin standard solutions are shown in Table 11. The plot of mangostin

concentrations versus the peak area ratios of mangostin and its internal standard, clotrimazole illustrated the linear correlation in the concentration range studied of 1-40 μg/ml (Figure 38). The coefficient of determination (R^2) of this line was 0.9999. These results indicated that HPLC method was acceptable for quantitative analysis of mangostin in the range studied.

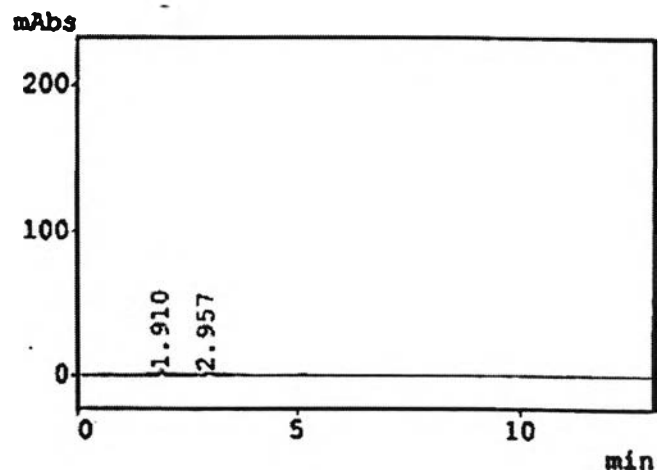


Figure 32 HPLC Chromatogram of blank solution (mobile phase).

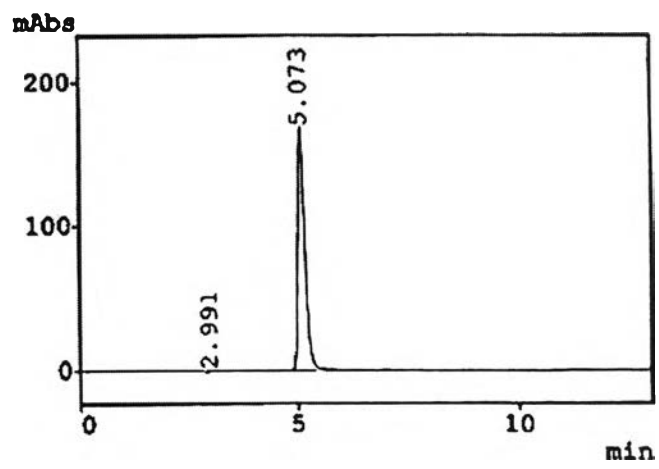


Figure 33 HPLC chromatogram of internal standard solution (clotrimazole).

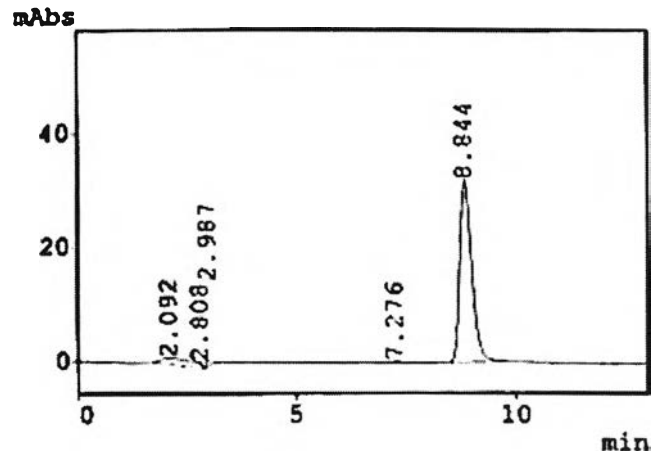


Figure 34 HPLC chromatogram of mangostin standard solution.

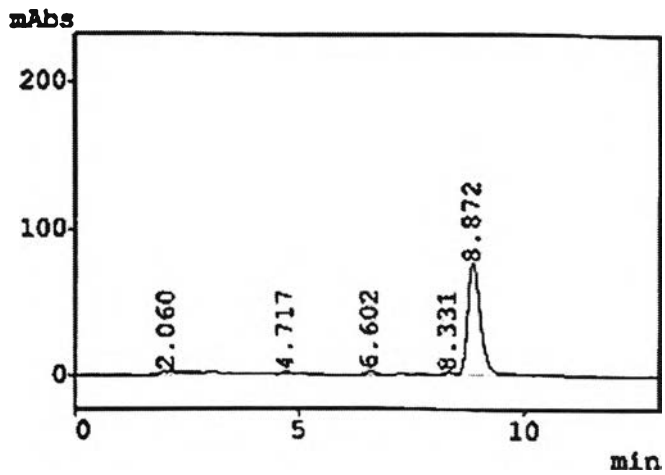


Figure 35 HPLC chromatogram of *Garcinia mangostana* extract solution.

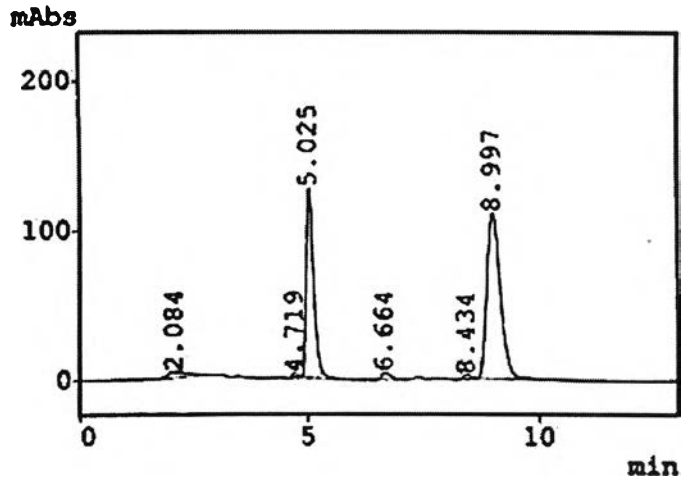


Figure 36 HPLC chromatogram of mixture of the extract and internal standard.

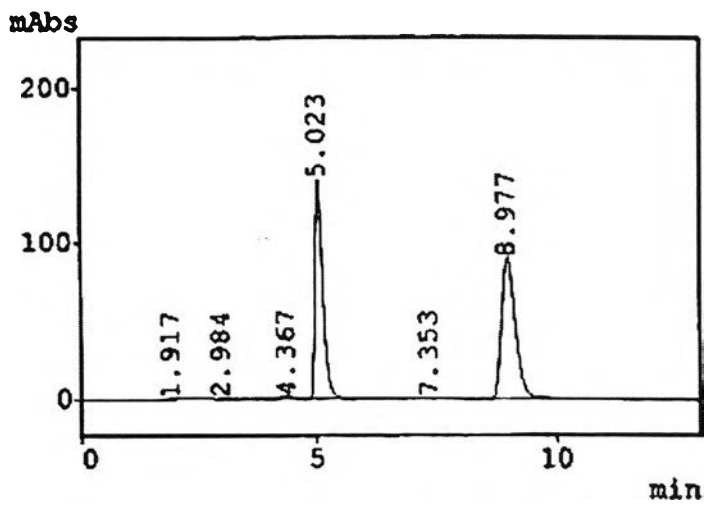


Figure 37 HPLC chromatogram of mixture of mangostin and internal standard.

Table 11 Data for calibration curve of mangostin by HPLC method

Concentration (μg/ml)	Peak area ratio			Mean	SD.	%CV
	Set 1	Set 2	Set 3			
1	0.0663	0.0661	0.0668	0.0664	0.0004	0.53
5	0.3028	0.3038	0.3070	0.3045	0.0022	0.72
10	0.6034	0.6102	0.6011	0.6049	0.0047	0.78
15	0.9078	0.8935	0.9010	0.9008	0.0071	0.79
20	1.1858	1.2121	1.1948	1.1976	0.0134	1.12
30	1.7783	1.7582	1.7598	1.7654	0.0111	0.63
40	2.3961	2.3828	2.3906	2.3899	0.0067	0.28
R^2	0.9999	0.9998	0.9997	0.9999	-	-

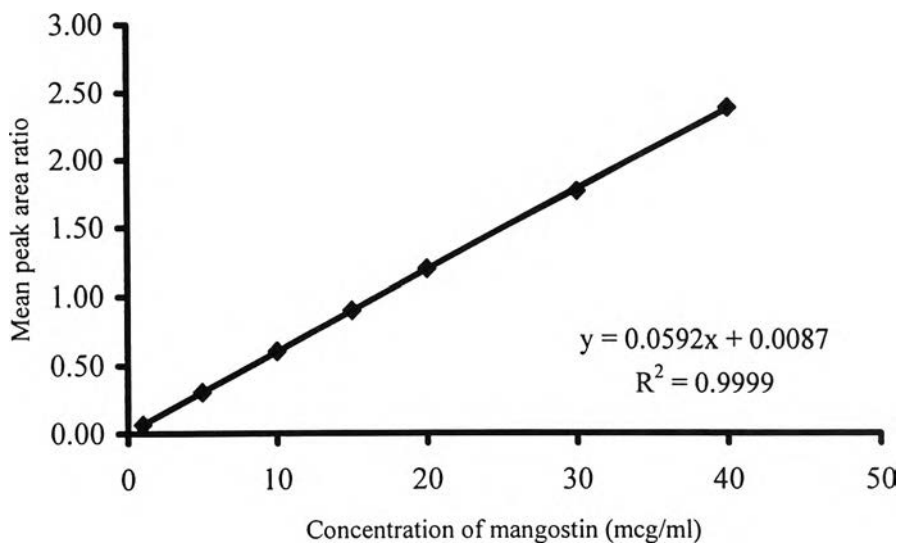


Figure 38 Calibration curve of mangostin by HPLC method

3.1.3 Accuracy

The determination of accuracy was performed by analyzing five sets of three concentration (8.0, 25.0, 35.0 μg/ml). The inversely estimated concentration and percentages of analytical recovery of each drug concentration are shown in Table 12 and Table 13, respectively. All percentages of analytical recovery were in the

range of 98.82 – 100.42 %, which indicated that this method could be used for analysis of mangostin in all concentrations studied with high accuracy.

Table 12 The inversely estimated concentrations of mangostin by HPLC method

Concentration (μg/ml)	Inversely estimated concentration (μg/ml)					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
8	7.9687	8.0675	7.9516	8.1124	8.0675	8.0335±0.07
25	24.7167	24.7683	24.8054	24.5531	24.6809	24.7049±0.10
35	34.6363	34.6929	34.8002	34.5115	34.6929	34.6668±0.11

Table 13 The percentage of analytical recovery of mangostin by HPLC method

Concentration (μg/ml)	% Analytical recovery					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
8	99.61	100.84	99.39	101.40	100.84	100.42±0.87
25	98.87	99.07	99.22	98.21	98.72	98.82±0.39
35	98.96	99.12	99.43	98.60	99.12	99.05±0.30

3.1.4 Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. The precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurements.

Table 14 and Table 15 illustrate the data of within run precision and between run precision, respectively. All coefficient of variation values were small, 0.32-0.85% and 0.66-1.43%, respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the HPLC method was precise for quantitative analysis of mangostin in the range studied.

Table 14 Data of within run precision by HPLC method

Concentration (μg/ml)	Peak area ratio					Mean	SD	%CV
	Set 1	Set 2	Set 3	Set 4	Set 5			
8	0.4804	0.4863	0.4794	0.4890	0.4863	0.4843	0.00	0.85
25	1.4719	1.4750	1.4772	1.4622	1.4698	1.4712	0.01	0.39
35	2.0592	2.0625	2.0689	2.0518	2.0625	2.0610	0.01	0.30

Table 15 Data of between run precision by HPLC method

Concentration (μg/ml)	Peak area ratio					Mean	SD	%CV
	Set 1	Set 2	Set 3	Set 4	Set 5			
8	0.4876	0.4783	0.4696	0.4796	0.4734	0.4777	0.01	1.43
25	1.4648	1.4785	1.4832	1.4688	1.4595	1.4710	0.01	0.66
35	2.0946	2.0523	2.0548	2.0685	2.0536	2.0648	0.02	0.87

In conclusion, the analysis of mangostin by HPLC method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for the determination of the content of mangostin in this study.

C. Determination of Solubility of *Garcinia mangostana* Extract

The solubility of *Garcinia mangostana* extract in hydroalcoholic mixtures at various concentrations of ethanol at ambient temperature are shown in Table 16. The solubility values expressed were the mean ± S.D. of three determinations. The sharp increase in the solubility of extract was observed as increasing ethanol content as cosolvent to the medium (Figure 39).

Table 16 The solubility of *Garcinia mangostana* extract

Solvent	Solubility (mg/ml) ± SD
40% ethanol in water	2.5399 ± 0.33
50% ethanol in water	9.5396 ± 0.65
60% ethanol in water	25.0200 ± 0.98
70% ethanol in water	85.5552 ± 2.84
80% ethanol in water	205.2323 ± 3.05

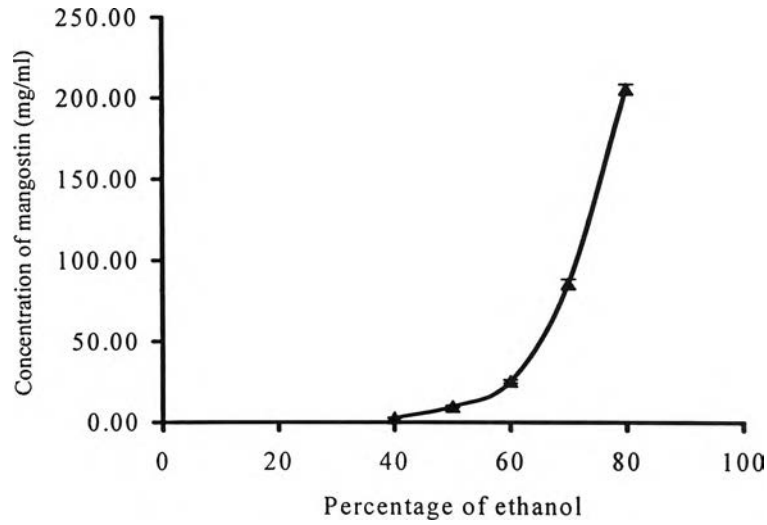


Figure 39 Solubility of mangostin of *Garcinia mangostana* extract in various concentrations of ethanol

1. Validation of UV spectrophotometric method

The validation of analytical method is the process for evaluation that the method is suitable and reliable for the intended analytical applications. The analytical parameters used for the UV spectrophotometric assay validation were specificity, linearity, accuracy and precision.

1.1 Specificity

The UV validation absorption spectrum of mangostin is shown the maximum absorbance at the wavelength of 243 nm. Therefore, the detection of mangostin was performed at this wavelength (Hiranras, 2001).

1.2 Linearity

The calibration curve of mangostin in absolute ethanol was shown in Figure 40. Linear regression analysis of the absorbances versus the corresponding concentrations was performed and the coefficient of determination (R^2) was calculated as 0.9999. The calibration data were found to be linear with excellent coefficient of determination. These results indicated that UV spectrophotometric method was acceptable for quantitative analysis of mangostin in the range studied.

Table 17 Data for calibration curve of mangostin by UV spectrophotometric method

Concentration (μg/ml)	Absorbance			Mean	SD.	%CV
	Set 1	Set 2	Set 3			
2.4	0.189	0.186	0.182	0.186	0.004	1.89
3.6	0.283	0.283	0.281	0.282	0.001	0.41
4.8	0.368	0.372	0.379	0.373	0.006	1.49
6.0	0.459	0.462	0.471	0.464	0.006	1.35
7.2	0.551	0.558	0.562	0.557	0.006	1.00
8.4	0.643	0.645	0.647	0.645	0.002	0.31
9.6	0.733	0.736	0.739	0.736	0.003	0.41
R^2	0.9999	0.9999	0.9994	0.9999	-	-

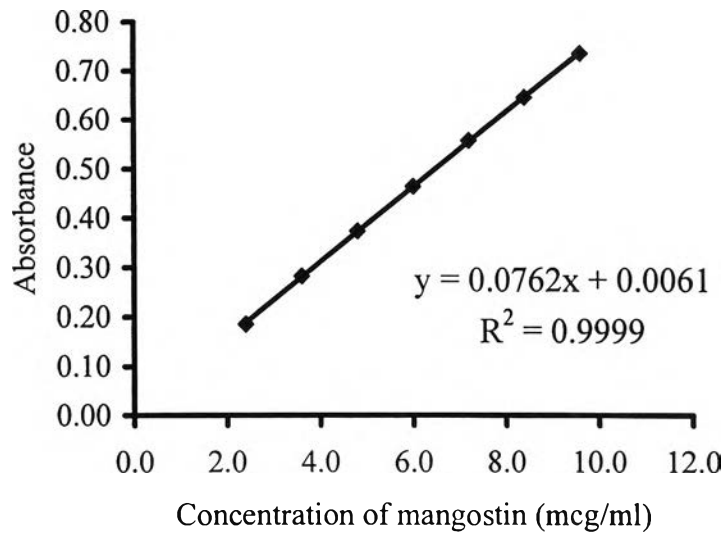


Figure 40 Calibration curve of mangostin by UV spectrophotometric method.

1.3 Accuracy

Mangostin solutions were prepared at the concentration of 3.0, 5.4, 7.8 μg/ml in five sets. Each individual sample was analyzed by UV spectrophotometer. The inversely estimated concentrations and percentages of analytical recovery of each drug concentration are shown in Table 18 and 19, respectively. All percentages of analytical recovery were in the range of 98.54-99.45%, which indicated the high

accuracy of this method. Thus, it could be used for analysis of mangostin in all concentrations studied.

Table 18 The inversely estimated concentrations of mangostin by UV spectrophotometric method

Concentration (μg/ml)	Inversely estimated concentration (μg/ml)					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
3.0	2.9922	2.9778	2.9490	2.9974	2.9895	2.9812 ± 0.02
5.4	5.4092	5.3922	5.3490	5.4026	5.2980	5.3702 ± 0.05
7.8	7.6693	9.6915	7.6967	7.6641	7.7085	7.6860 ± 0.02

Table 19 The percentage of analytical recovery of mangostin by UV spectrophotometric method

Concentration (μg/ml)	% analytical recovery					Mean ± SD
	Set 1	Set 2	Set 3	Set 4	Set 5	
3.0	99.74	99.26	98.30	99.91	99.65	99.37 ± 0.65
5.4	100.17	99.85	99.06	100.05	98.11	99.45 ± 0.86
7.8	98.32	98.61	98.68	98.26	98.83	98.54 ± 0.24

1.4 Precision

The precision of mangostin analyzed by UV spectrophotometric method were determined both within run precision and between run precision as illustrated in Tables 20-21. All coefficients of variation values were very low, as 0.24-0.87% and 0.62-1.13%, respectively. The coefficient of variation of an analytical method should generally be less than 2%. Therefore, the UV spectrophotometric method was precise for quantitative analysis of mangostin in the range studied.

Table 20 Data of within run precision by UV spectrophotometric method

Concentration (μg/ml)	Absorbance					Mean	SD	%CV
	Set 1	Set 2	Set 3	Set 4	Set 5			
3	0.2294	0.2283	0.2261	0.2298	0.2292	0.2286	0.001	0.65
5.4	0.4143	0.4130	0.4097	0.4138	0.4058	0.4109	0.004	0.87
7.8	0.5872	0.5889	0.5893	0.5868	0.5902	0.5885	0.001	0.24

Table 21 Data of between run precision by UV spectrophotometric method

Concentration (μg/ml)	Absorbance					Mean	SD	%CV
	Set 1	Set 2	Set 3	Set 4	Set 5			
3	0.2255	0.2248	0.2296	0.2231	0.2278	0.2262	0.003	1.13
5.4	0.413	0.4155	0.4201	0.4119	0.4162	0.4153	0.003	0.77
7.8	0.5877	0.5921	0.5861	0.5823	0.5848	0.5866	0.004	0.62

In conclusion, the analysis of mangostin in by UV spectrophotometric method developed in this study showed good specificity, linearity, accuracy and precision. Thus this method was used for the determination of the content of mangostin in the solubility study.

D. Determination of Antimicrobial Activities of *Garcinia mangostana* Extract

1. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The experiment was performed with three series of extract. After incubation the MIC was determined visually as the lowest concentration that inhibited bacterial growth, which was demonstrated by the absence of turbidity. The MIC of the extract 3 μg/ml for *Staphylococcus aureus* ATCC 25923 and 1.5 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$ (Table 22).

The MBC was determined the lowest concentration of an antimicrobial agent that killed the test bacteria. The MBC of the extract was 4 μg/ml for *Staphylococcus aureus* ATCC 25923 and 3 μg/ml for *Streptococcus mutans* ATCC $KPSK_2$ (Table 22).

From the study, it was interesting that the MIC and MBC values of the extract against *Streptococcus mutans* ATCC $KPSK_2$ were lower than that against *Staphylococcus aureus* ATCC 25923. This result implied that *Garcinia mangostana*

extract had a higher activity against *Streptococcus mutans* ATCC $KPSK_2$ than against *Staphylococcus aureus* ATCC 25923. This finding is consistent to the previous study reported by Hiranras (2001).

2. Determination of solvent effect on bacterial growth inhibition

From the previous study, it was found that mangostin has poor solubility in water and high solubility in ethanol (Budavari, 2001; Hiranras, 2001). Thus, in this present work, ethanol was selected to solubilize the extract. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol against *Staphylococcus aureus* ATCC 25923 was 5.9375% v/v and 23.75% v/v, respectively. The minimum concentration of ethanol that showed the inhibitory effect against *Streptococcus mutans* ATCC $KPSK_2$ was found to be 11.875% v/v and showed bactericidal effect at 23.75% v/v. The MIC and MBC of ethanol against two types of tested bacteria was higher than of the extract. These results showed that the ethanol content of the extract solution in this experiment did not interfere the MIC and MBC of the extract.

Table 22 The MIC and MBC of extract and ethanol on various types of bacteria.

Types of bacteria	MIC (μg/ml)	MBC (μg/ml)	MIC of ethanol (%v/v)	MBC of ethanol (%v/v)
Staphylococcus aureus ATCC 25923	3	4	5.9375	23.75
Streptococcus mutans $KPSK_2$	1.5	3	11.875	23.75

E. Formulation of Orally Fast Dissolving Films Base

From the preliminary studies, appropriated type and concentration of cellulose polymers were investigated to obtain fast dissolving films with satisfactory appearance, good integrity, and which were easily detached from the glassy plate after

drying with flexibility and no breakage. Consequently, three types of cellulose were employed for the study including HPMC 3 cps, HPMC 5 cps, and HPC.

1. *In vitro* evaluation of orally fast dissolving films

1.1 Physical characteristics of orally fast dissolving film bases

In this experiment, the films were prepared by solvent casting method on glassy plates. The films were cut into 2 x 3 cm that appropriate for place on the tongue (Xu et al., 2002; Szeles et al., 2004). The physical characteristics of orally fast dissolving films were transparent, glossy, flexible, sticky and easy to peel off from the glass flat plate as shown in Table 23.

From the result, it was found that the film formula that consisted of 3 plasticizers, PEG 400, PG and glycerin at 15% total amount of plasticizers per total amount of polymers was too moist and soft that caused self-adhering characteristic. It might be that all plasticizers are polyol substances, thus they have moisture absorption characteristic. Additionally, the film formulations already incorporated volatile oil which may soften and moisten the film. This finding is consistent with a previous study that the pullulan film products containing sorbitol, glycerin or both were easily broken into pieces, or could be too moist and/or self-adhering but they could produce films that rapidly dissolved in the oral cavity (Mcgregor, Homan, and Gravina, 2004).

The characteristics of film consisting of only HPC as film former were translucent, too soft, highly sticky and difficult to detach from flat glass plate but they were flexible. While the HPMC film was transparent and easy to peel. Therefore, for improving ease of peeling of HPC film, the combination of film formers between HPC and HPMC could provide the films that were easier to detach than HPC film alone. These findings agree with the previous study by Taweekunthum (2001), that used the combined polymers between HPMC 15 cps and HPC HV (high viscosity).

Focus on HPMC films, this study found that the film prepared from HPMC 5 cps provided more transparent and colorless than one from HPMC 3 cps. It may be due to the yellow-white color of HPMC 3 cps powder. Additionally, HPMC 5 cps (E5) film was more flexible than HPMC 3 cps (E3) film.

Table 23 Physical characteristics of fast dissolving film bases

Formulas	Transparency	Glossiness	Flexibility	Stickiness	Ease of peeling
PEG/PG	++	++	+++	++	+++
PEG/GLY	++	++	++	++	+++
PEG/PG/GLY	++	++	++	+++	+++
PEG	++	++	++	+	+++
PG/GLY	++	++	++	++	+++
GLY	++	++	++	+	+++
PG	++	++	+++	+	+++
E3	++	++	+	+	+++
E5	+++	+++	+++	+	+++
HPC	+	+	+++	+++	+
E3HPC (2:1)	++	++	+++	+	+++
E3HPC (3:1)	++	++	+++	+	+++
E3HPC (4:1)	++	++	+++	+	+++
E3HPC (5:1)	++	++	+++	+	+++
E3E5 (1:1)	++	+++	+++	+	+++
E3E5 (2:1)	++	+++	+++	+	+++
E3E5 (3:1)	++	+++	+++	+	+++
E3E5 (5:1)	++	+++	+++	+	+++
E5HPC (1:1)	++	+++	+++	++	++
E5HPC (2:1)	++	+++	+++	+	+++
E5HPC (3:1)	++	+++	+++	+	+++
E5HPC (5:1)	++	+++	+++	+	+++

The symbols of (+) and (-) mean the appearance and no appearance, respectively. The number of the symbols of (+) means a degree of the appearance of the specified property.

1.2 Thickness of orally fast dissolving films

There are many studies that discuss the thickness of fast dissolving or breath freshening films. Many studies reported that the appropriate thickness of the fast dissolving film should be in range from 35 to 45 μm and not more than 70 μm (Marco and Fausto, 2004). While another patent claimed that the film preferably has a thickness about 40-60 μm (Szeles et al., 2004). Additionally, Xu et al. (2002) reported

that the film has a thickness ranging from about 15 to about 80 μm, and preferably about 30 to 60 μm. The results from the thickness measurement using micrometer (Starrett, USA) are presented in appendix C and the average thickness of the prepared orally fast dissolving film bases are given in Table 24.

Table 24 The average thickness of the prepared fast dissolving film bases (n=5, each sample was measured at 5 locations).

Formulas	Average thickness ± SD. (μm)					Mean
	No. 1	No. 2	No. 3	No. 4	No. 5	± SD
PEG/PG	40.2±1.30	40.0±1.22	37.8±2.17	37.8±1.92	40.2±0.45	39.2±1.28
PEG/GLY	41.2±2.59	42.6±1.95	39.0±2.45	41.2±1.79	39.2±1.30	40.6±1.52
PEG/PG/GLY	44.8±1.30	50.4±2.61	48.8±1.30	49.0±1.58	46.0±2.55	47.8±2.32
PG	41.8±1.48	39.4±1.14	38.4±1.14	39.0±1.87	38.4±1.14	39.4±1.41
PG/GLY	39.2±1.64	38.2±1.30	35.4±0.55	37.4±2.51	37.8±0.84	37.6±1.40
GLY	35.6±0.55	36.0±0.71	36.2±0.84	34.6±0.55	38.0±1.00	36.1±1.24
PEG	34.0±0.00	33.6±0.55	32.4±2.19	33.2±1.10	35.8±2.84	33.8±1.26
E3	38.6±1.82	40.6±2.19	39.6±1.95	42.8±1.30	42.2±1.48	40.8±1.75
E5	38.0±0.71	37.8±0.84	38.0±1.00	40.0±1.41	40.4±1.34	38.8±1.25
HPC	39.6±2.88	38.2±1.92	39.6±2.07	39.6±1.67	40.2±1.30	39.4+0.74
E3HPC (2:1)	36.2±0.84	38.4±0.55	39.2±0.84	38.4±1.14	37.8±0.45	38.0±1.12
E3HPC (3:1)	35.2±0.45	35.4±0.55	39.8±1.30	38.0±1.00	38.4±0.55	37.4±2.00
E3HPC (4:1)	35.0±0.00	34.6±0.55	34.0±0.71	34.2±0.84	35.0±2.35	34.6±0.46
E3HPC (5:1)	37.8±1.48	35.8±0.84	35.4±1.14	34.8±0.84	36.4±0.55	36.0±1.14
E3E5 (1:1)	37.0±1.10	37.2±1.00	37.8±1.10	38.4±0.55	36.6±1.52	37.4±0.71
E3E5 (2:1)	34.0±0.71	36.4±0.55	35.0±0.71	38.0±1.58	34.8±1.48	35.6±1.58
E3E5 (3:1)	40.6±0.55	34.8±0.45	35.8±1.30	36.6±1.67	37.0±1.00	37.0±2.20
E3E5 (5:1)	38.0±0.00	37.6±1.14	38.6±1.52	36.2±2.17	35.4±1.14	37.2±1.32
E5HPC (1:1)	36.4±0.55	36.4±2.30	35.8±1.10	38.8±1.30	36.4±0.55	36.8±1.17
E5HPC (2:1)	36.6±1.14	36.0±1.22	35.8±1.30	41.0±0.00	36.8±0.45	37.2±2.14
E5HPC (3:1)	36.8±0.45	36.0±1.22	35.6±0.89	37.6±0.55	37.8±1.30	36.8±0.96
E5HPC (5:1)	32.2±0.45	33.8±0.45	33.0±1.22	33.2±0.84	32.6±0.55	33.0±0.61
Commercial product strips A	41.2±1.79	37.4±1.34	40.4±2.07	43.8±1.48	41.0±2.12	40.8±2.29

1.3 Weight variation of orally fast dissolving films

Many patents claimed that the weight of orally fast dissolving films should be in the range of 30 to 100 mg (Pearce, 2003) but another patent claimed that weight variation should be between 25 and 35 mg (Marco and Fausto, 2004). The results of weight variation are presented in Appendix C. It shows in Table 25 shows the average weights of all test films which ranged from 20.18±0.50 to 31.60±1.62 mg.

Table 25 Weight variation of orally fast dissolving films (n=5).

Formulas	Weight (mg) ± SD	%CV
PEG/PG	26.46 ± 0.11	4.20
PEG/GLY	25.90 ± 0.75	2.91
PEG/PG/GLY	31.60 ± 1.62	5.13
PG	24.34 ± 0.62	2.53
PEG	21.92 ± 0.97	4.42
PG/GLY	24.22 ± 1.66	6.85
GLY	24.08 ± 1.78	7.37
E3	26.91 ± 1.21	4.48
E5	23.53 ± 1.72	7.31
HPC	20.18 ± 0.50	2.46
E3HPC (2:1)	23.43 ± 0.29	1.24
E3HPC (3:1)	22.67 ± 1.46	6.43
E3HPC (4:1)	21.46 ± 1.12	5.23
E3HPC (5:1)	23.61 ± 1.04	4.43
E3E5 (1:1)	25.88 ± 0.83	3.19
E3E5 (2:1)	23.71 ± 1.22	5.15
E3E5 (3:1)	25.43 ± 1.96	7.70
E3E5 (5:1)	24.42 ± 1.80	7.35
E5HPC (1:1)	23.65 ± 0.86	3.63
E5HPC (2:1)	25.17 ± 1.54	6.11
E5HPC (3:1)	25.04 ± 0.55	2.20
E5HPC (5:1)	21.90 ± 0.25	1.13
Commercial product strips A	28.83 ± 0.77	2.67

1.4 Mechanical properties of orally fast dissolving films

Mechanical properties including percent strain at point of break, Young's modulus, ultimate tensile strength and work of failure are presented in Table 26 and Figures 41-48.

Focused on percent strain at point break, the results showed that the film from HPC had the high percent strain at break value. This represented to the elasticity characteristic but it was too soft when focused in tensile strength. This finding is consistent with the tensile property study that it had very low tensile strength and Young's modulus indicating it was soft, tough and weak (Taweekunthum, 2001).

The film consisted of HPMC 3 cps with 3 plasticizers including PEG 400, PG, and glycerin showed the lowest tensile strength and work of failure comparing with the HPMC 3 cps film without any plasticizer and with one or two types of plasticizers. This data supported the physical characteristics of this film that these polyol type plasticizers made the film too soft and easily broke into pieces.

Focused on the films that were combination of HPMC 5 cps and HPC LV, it was found that the ultimate tensile strength and Young's modulus values increased as increasing the proportion of HPMC 5 cps in the films. Inversely, they showed decreasing of percentage of elongation significantly ($p<0.05$). Therefore, the combination of HPMC 5 cps and HPC LV as film formers provided the films that were more stronger and rigid than HPC alone.

While combining film with HPMC 3 cps and HPC LV exhibited increasing of tensile strength and Young's modulus, percent elongation at break and work of failure decreased. These results indicated that mixed polymers of HPMC 3 cps and HPC LV were more hard, rigid and brittle than HPC film. In addition, all ratios of these mixed polymers gave no remarkable difference in percent elongation at break of films ($p>0.05$).

The films that consisted of HPMC 3 cps and HPMC 5 cps showed sharply decreasing trend of tensile strength, percent elongation and work of failure when

proportion of HPMC 3 cps increased. This finding revealed that the amount of HPMC 3 cps have a great effect on mechanical properties of films of HPMC 5 cps and 3 cps combination.

Table 26 Mechanical properties of orally fast dissolving films (n=5).

Formulas	Tensile strength ±SD (MPa)	% Strain at point of break ± SD (%)	Young's modulus ± SD (MPa)	Work of failure ± SD.(mJ)
PEG/PG	40.84 ± 1.24	16.57 ± 2.16	385.60 ± 15.20	1.65 ± 0.36
PEG/GLY	35.26 ± 1.50	11.91 ± 0.78	374.94 ± 9.90	0.86 ± 0.12
PEG/PG/GLY	24.70 ± 0.83	12.90 ± 1.00	323.14 ± 7.19	0.70 ± 0.12
PG	47.34 ± 1.59	19.76 ± 1.31	386.02 ± 10.58	2.38 ± 0.23
PG/GLY	34.46 ± 1.41	14.59 ± 1.21	344.96 ± 14.92	1.31 ± 0.23
GLY	52.24 ± 0.98	27.37 ± 0.44	412.12 ± 11.47	3.99 ± 0.23
PEG	46.53 ± 1.69	18.98 ± 1.41	376.74 ± 25.08	2.00 ± 0.37
E3	43.59 ± 3.82	14.35 ± 0.51	431.50 ± 14.96	1.23 ± 0.14
E5	72.38 ± 8.04	254.42 ± 4.76	442.14 ± 16.68	52.84 ± 7.86
HPC	10.43 ± 0.75	59.00 ± 1.96	179.56 ± 15.82	2.11 ± 0.14
E3HPC (2:1)	42.86 ± 1.61	14.90 ± 1.03	405.36 ± 10.26	1.21 ± 0.15
E3HPC (3:1)	45.17 ± 3.91	14.50 ± 0.98	397.40 ± 28.17	1.24 ± 0.27
E3HPC (4:1)	47.53 ± 0.77	15.04 ± 0.42	442.28 ± 9.08	1.23 ± 0.07
E3HPC (5:1)	47.13 ± 2.84	13.74 ± 1.23	443.80 ± 12.57	1.19 ± 0.18
E3E5 (1:1)	65.06 ± 4.03	220.84 ± 9.15	463.02 ± 17.20	46.13 ± 5.88
E3E5 (2:1)	59.40 ± 2.04	105.20 ± 7.06	440.22 ± 13.48	22.48 ± 7.42
E3E5 (3:1)	63.72 ± 3.16	53.32 ± 10.37	468.32 ± 24.83	9.64 ± 2.09
E3E5 (5:1)	54.80 ± 1.90	49.44 ± 4.16	433.06 ± 25.92	7.19 ± 0.99
E5HPC (1:1)	22.20 ± 0.36	18.02 ± 1.17	335.6 ± 22.30	1.03 ± 0.08
E5HPC (2:1)	32.75 ± 1.43	31.48 ± 1.45	379.9 ± 21.30	2.92 ± 0.25
E5HPC (3:1)	35.46 ± 0.57	27.12 ± 0.64	406.3 ± 13.49	2.57 ± 0.11
E5HPC (5:1)	49.10 ± 1.02	36.80 ± 1.67	452.12 ± 17.52	4.54 ± 0.25
Commercial product strips A	49.13 ± 1.42	16.74 ± 0.90	483.60 ± 10.07	1.97 ± 0.20

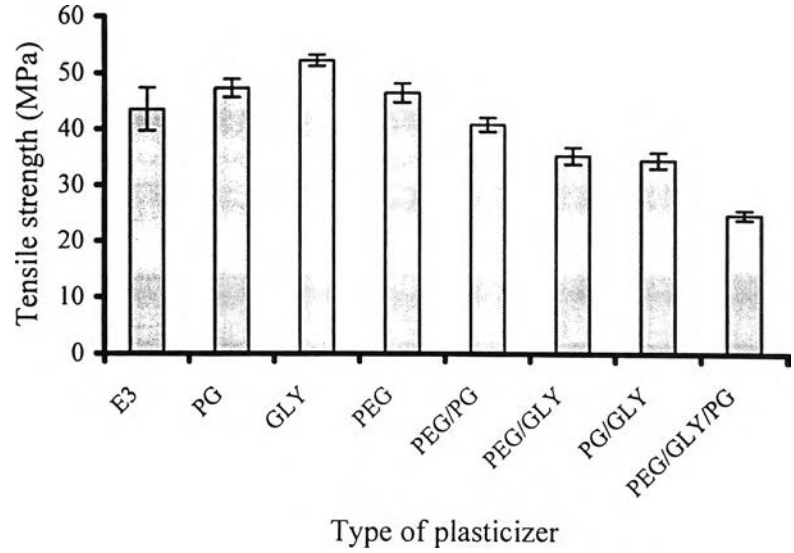


Figure 41 Tensile strength of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

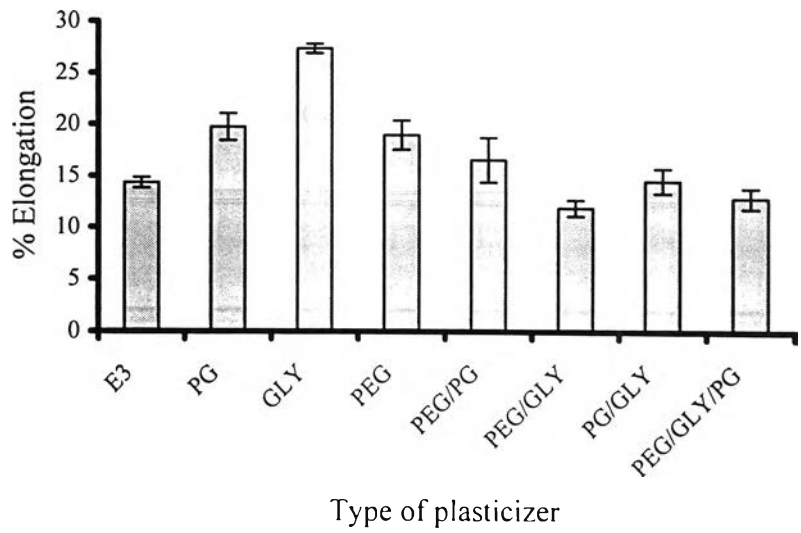


Figure 42 Percentage of elongation at break of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

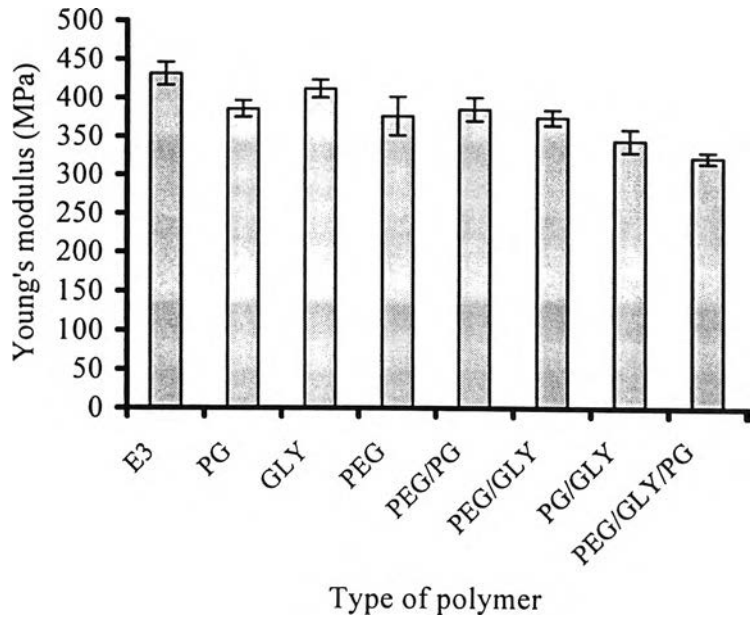


Figure 43 Young's modulus of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.

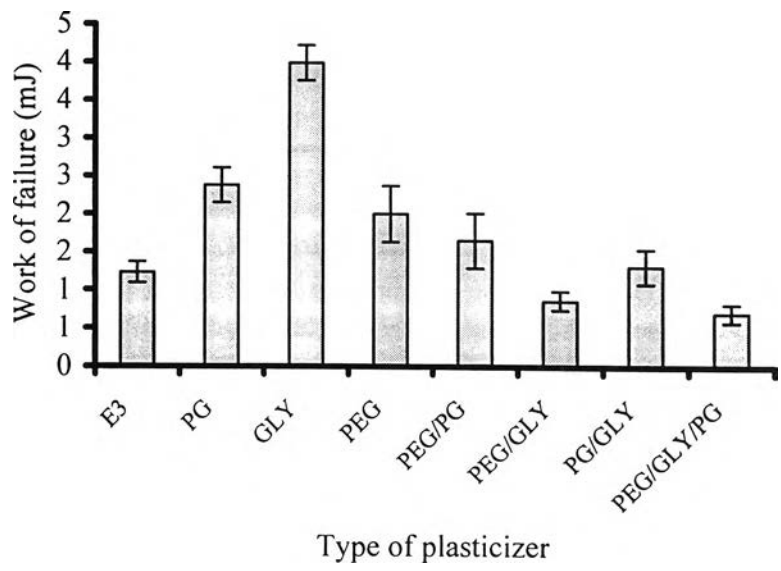
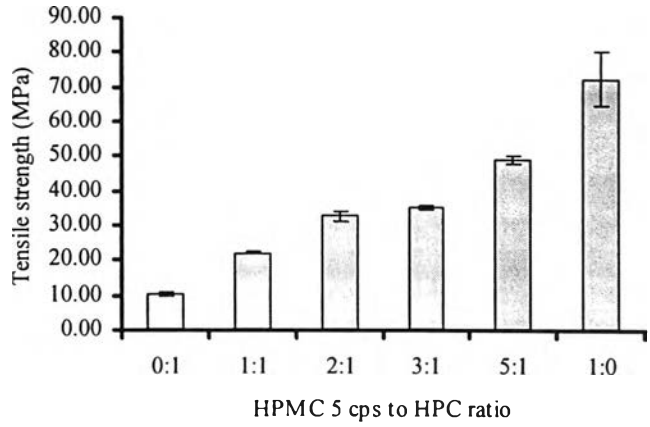
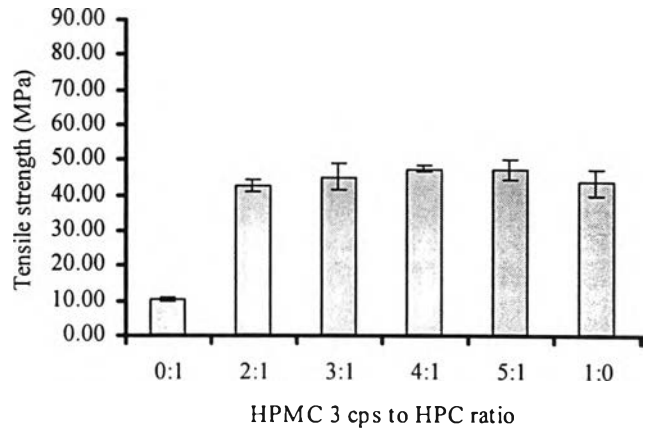


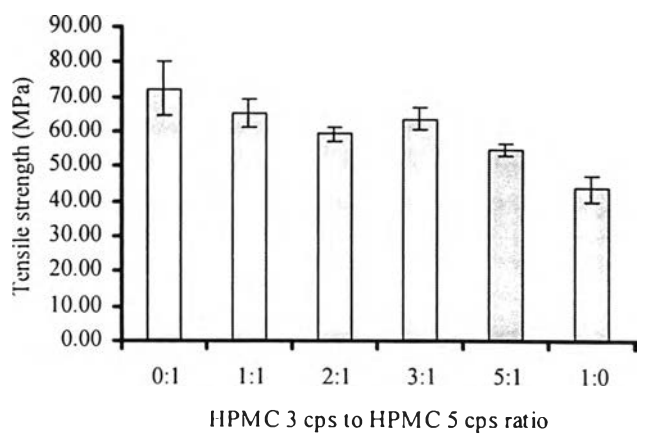
Figure 44 Work of failure of orally fast dissolving film bases in formulation of HPMC 3 cps with various plasticizers.



(a)

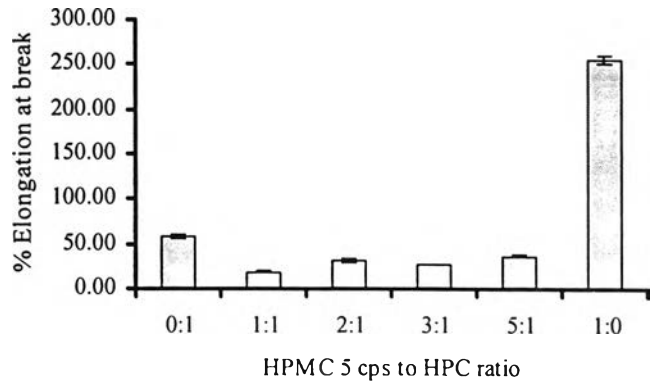


(b)

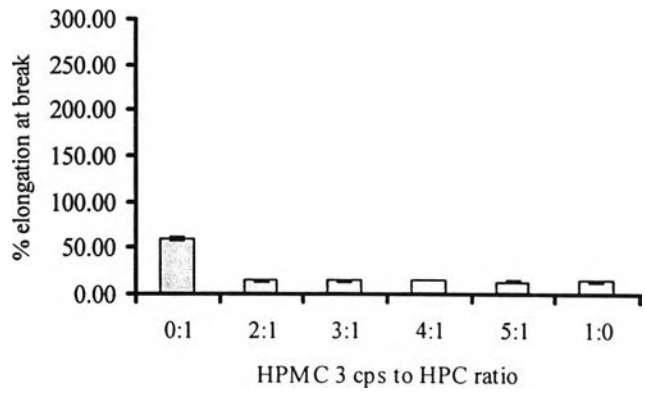


(c)

Figure 45 Tensile strength of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5



(a)



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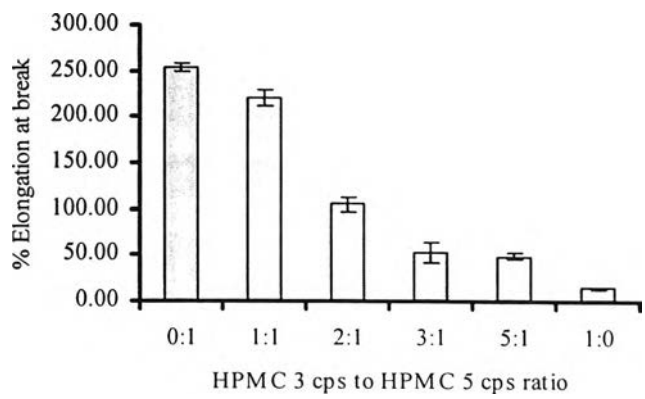
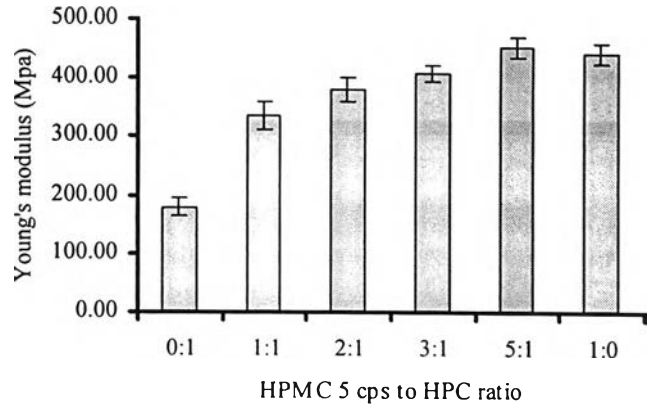
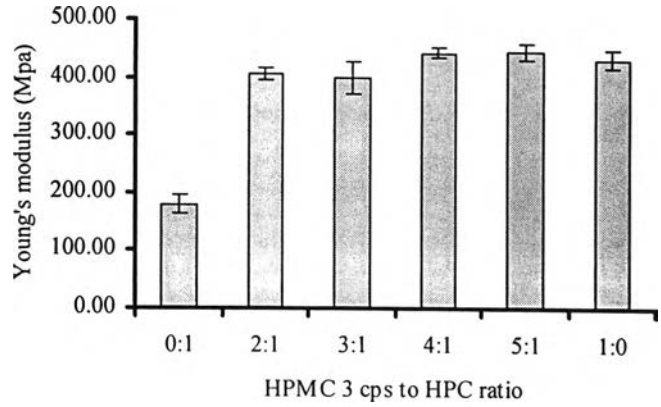


(c)

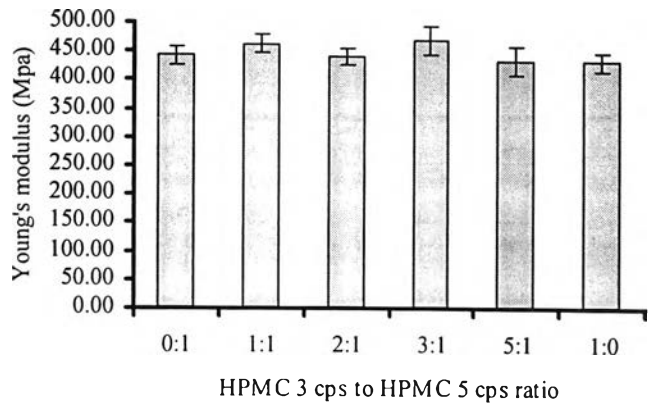
Figure 46 Percentage of elongation at break of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5



(a)

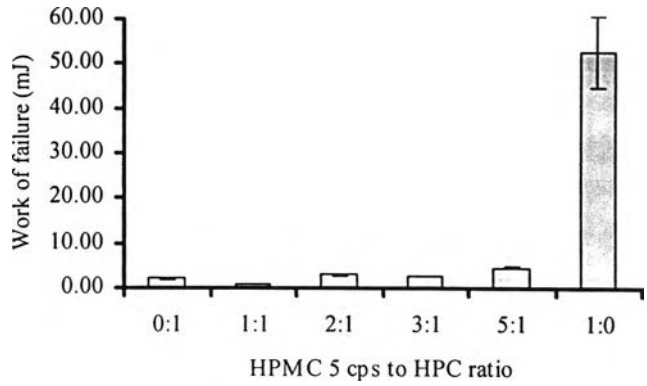


(b)

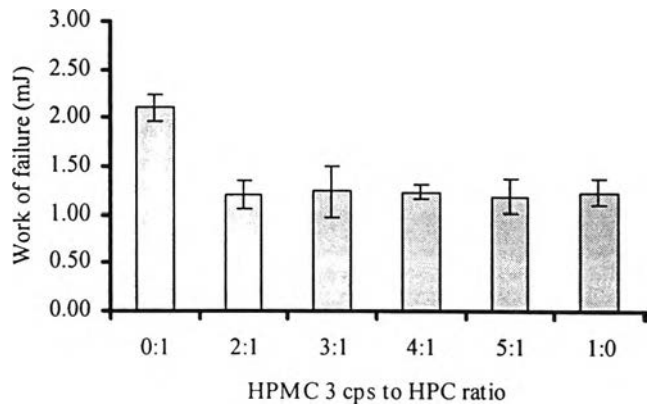


(c)

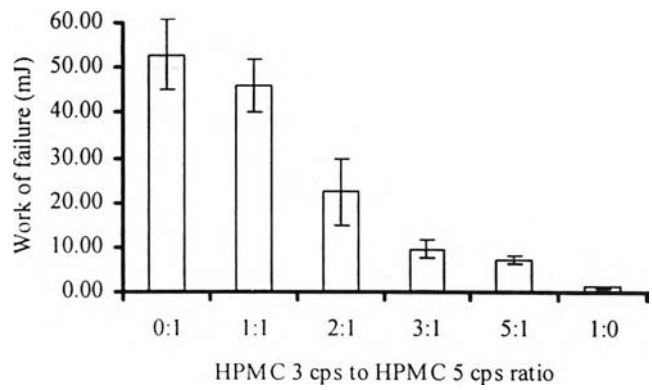
Figure 47 Young's modulus of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5



(a)



(b)



(c)

Figure 48 Work of failure of orally fast dissolving film bases in formulation of (a) E5HPC, (b) E3HPC and (c) E3E5

1.5 Dissolution time of orally fast dissolving films

The dissolution time of the film is the most important properties for customer or patient's acceptance and it is a critical criteria for selecting film base to formulate the film containing *Garcinia mangostana* extract. Xu et al.(2002) defined that the fast dissolving strips were quickly dissolved in less than 30-40 seconds but in another patent claimed that the films should dissolved or disintegrated completely within 60 seconds in oral cavity (Szeles et al., 2004). In this experiment, *in vitro* speed of dissolution of films was determined by a modified method from Xu et al.(2002), explaining that the dissolution time was the time which the film disappeared or completely disintegrated. The dissolution time study was performed in 5 replications.

In this study, commercial product strips A, the commercial fast dissolving breath strip, was used as control for selecting the film base to formulate the fast dissolving film containing *Garcinia mangostana* extract. It was found that all formulations of E3HPC were not significantly different in dissolution time from commercial product strips A ($p>0.05$).

The film formula that gave the fastest dissolution was HPC and the film which gave the latest dissolution time was HPMC 5 cps and HPMC 3 cps dissolved faster than HPMC 5 cps about 6 times. This finding is similar to the result from the previous investigation by Xu et al.(2002) that films prepared using high viscosity grade of HPMC dissolved appreciably slower than a film prepared using lower viscosity grade.

Table 27 Dissolution time of fast dissolving films (n=5).

Formulas	Dissolution time (sec)					Mean ± SD	% CV
	No. 1	No. 2	No. 3	No. 4	No. 5		
PEG/PG	191	186	191	167	173	181.6 ± 10.99	6.05
PEG/GLY	156	206	181	206	165	182.8 ± 22.99	12.58
PEG/PG/GLY	357	322	345	386	330	348.0 ± 25.17	7.23
PG	195	216	192	222	182	201.4 ± 16.91	8.39
PG/GLY	443	371	495	431	414	430.8 ± 45.08	10.46
GLY	280	266	330	320	328	304.8 ± 29.69	9.74
PEG	280	323	328	272	315	303.6 ± 25.77	8.49
E3	205	182	224	182	211	200.8 ± 18.49	9.21
E5	1278	1092	1228	1207	1270	1215.0 ± 74.76	6.15
HPC	73	80	64	53	55	65.0 ± 11.55	17.78
E3HPC (2:1)	133	118	129	123	104	121.4 ± 11.28	9.29
E3HPC (3:1)	82	100	99	130	126	107.4 ± 20.17	18.78
E3HPC (4:1)	83	90	123	88	128	102.4 ± 21.31	20.81
E3HPC (5:1)	125	131	141	139	130	133.2 ± 6.65	4.99
E3E5 (1:1)	218	232	205	352	245	250.4 ± 58.74	23.46
E3E5 (2:1)	143	200	179	191	158	174.2 ± 23.49	13.48
E3E5 (3:1)	161	188	202	204	219	194.8 ± 21.86	11.22
E3E5 (5:1)	197	285	179	195	190	209.2 ± 42.94	20.53
E5HPC (1:1)	168	204	213	149	141	175.6 ± 31.50	17.94
E5HPC (2:1)	267	193	197	211	201	213.8 ± 30.48	14.26
E5HPC (3:1)	200	249	320	200	316	257.0 ± 59.19	23.03
E5HPC (5:1)	235	269	265	160	305	246.8 ± 54.51	22.09
Commercial product strips A	110	109	132	158	96	121.0 ± 24.39	20.16

F. Formulation of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract

From the results of evaluation of film bases, the optimal formulas which appropriate properties such as good physical characteristic, suitable mechanical properties, and fast disintegrating time were selected to formulate orally fast dissolving films containing *Garcinia mangostana* extract. These formulas including E3HPC in proportion of 2:1, 3:1, 4:1, and 5:1 were selected. The concentration of extract employed in the formulations was about 0.15% w/w of dried film base. That is the film of 2 x 3 cm^2 contained an approximate weight of 181.61 μg of mangostin of extract. The physical properties of four formulations were depicted in Table 29. As a result, all formulations were clear and yellow color due to the color of extract. Other physical appearances of each formulation were similar to film bases.

Table 28 Formulation of orally fast dissolving films containing *Garcinia mangostana* extract

Composition	Formulation code (% w/w)			
	E3HPC 2:1	E3HPC 3:1	E3HPC 4:1	E3HPC 5:1
HPMC 3 cps	8	9	9.6	10
HPC LV	4	3	2.4	2
Acesulfame K	0.3	0.3	0.3	0.3
Menthol	1.5	1.5	1.5	1.5
Eucalyptus oil	0.6	0.6	0.6	0.6
Garcinia mangostana extract	0.216	0.216	0.216	0.216
60% ethanol	85.6	85.6	85.6	85.6

Table 29 Physical characteristics of orally fast dissolving films containing *Garcinia mangostana* extract

Formulas	Transparency	Glossiness	Flexibility	stickiness	Ease of peeling
E3HPC (2:1)	++	++	+++	+	+++
E3HPC (3:1)	++	++	+++	+	+++
E3HPC (4:1)	++	++	+++	+	+++
E3HPC (5:1)	++	++	+++	+	+++

The symbols of (+) and (-) show the appearance and no appearance, respectively. The number of the symbols of (+) means a degree of the appearance of the specified property.

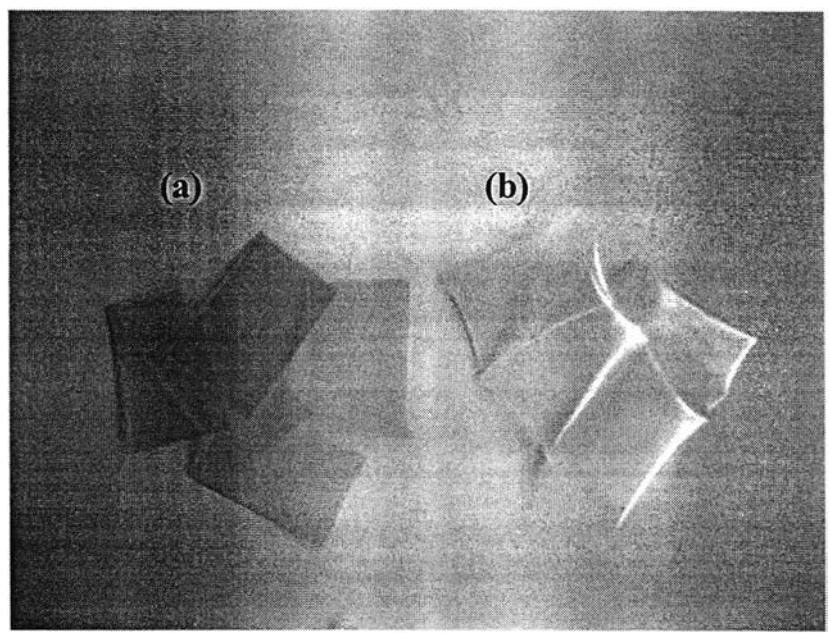


Figure 49 Orally fast dissolving films; (a) films containing *Garcinia mangostana* extract and (b) film bases

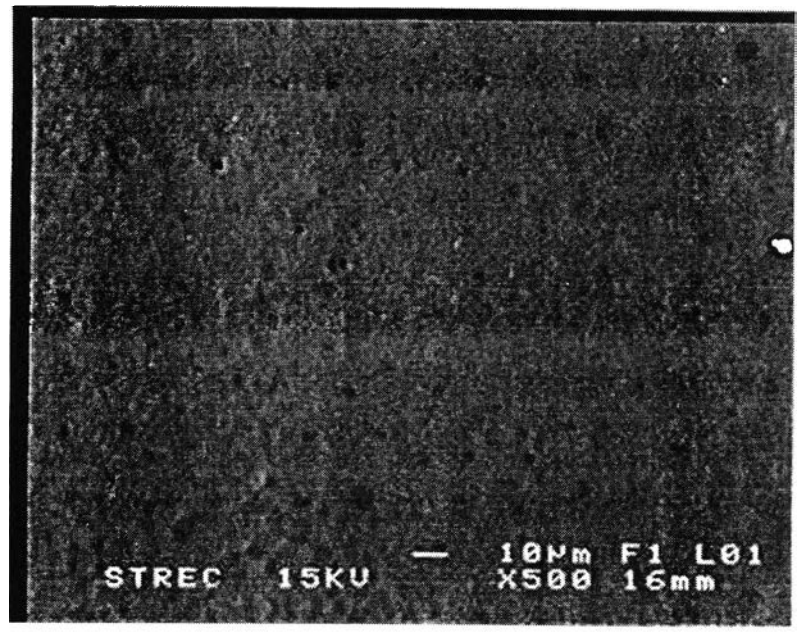
G. Characterization of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract

1. Surface morphology

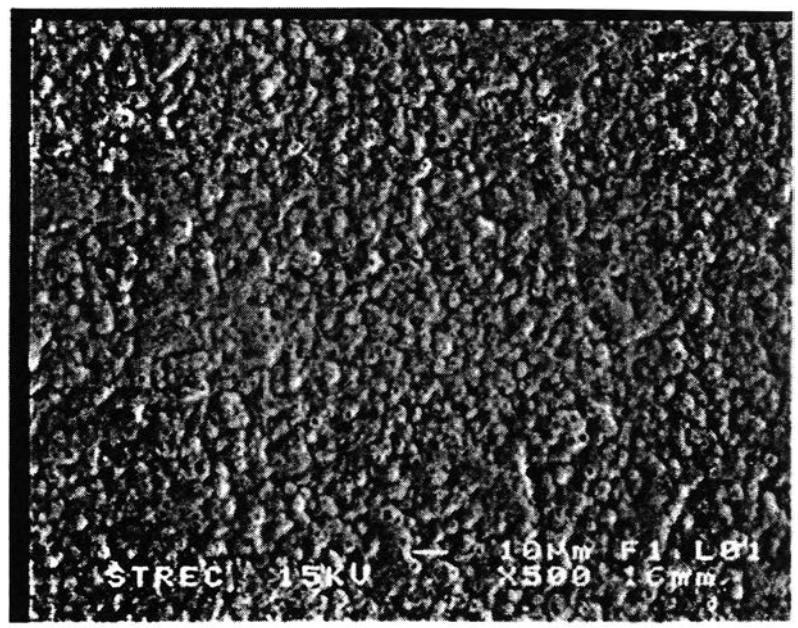
The surface morphology of the four formulations containing *Garcinia mangostana* extract was observed by using scanning electron microscope (SEM). From the photomicrographs of cross-sectional area of E3HPC film bases, it was found that E3HPC (3:1) film exhibited dense, smooth and homogeneous texture. These were more than other formulations of film base as seen in Figure 51 (a).

All formulations of the prepared films from combination of HPMC 3 cps and HPC LV showed rough surface with many pores. This result is consistent with the finding from Taweekunthum (2001) that observed surface topography of film consisted of HPMC 15 cps and HPC.

Comparison of fast dissolving film containing *Garcinia mangostana* extract with its film base in all formulations, it indicated that the incorporation of extract into films caused a slight increase of surface roughness and porosity (Figure 50-53).

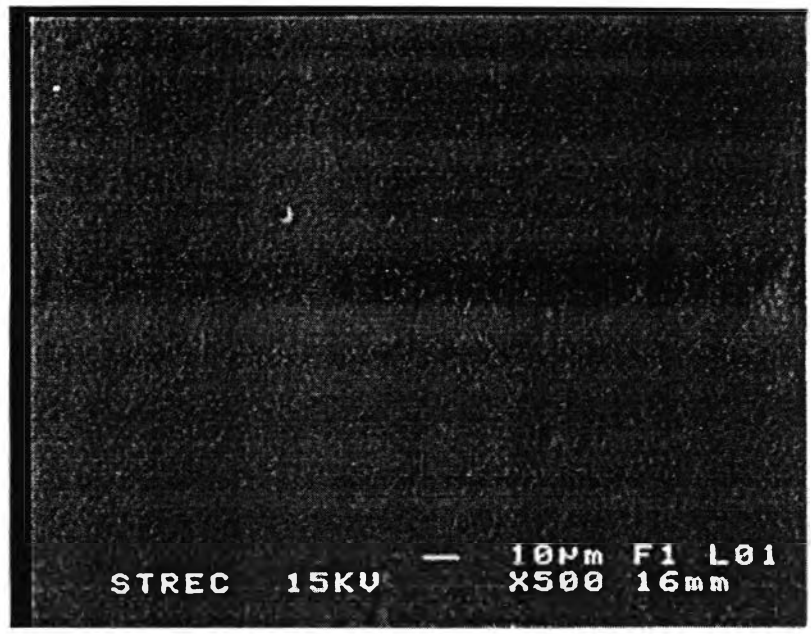


(a)

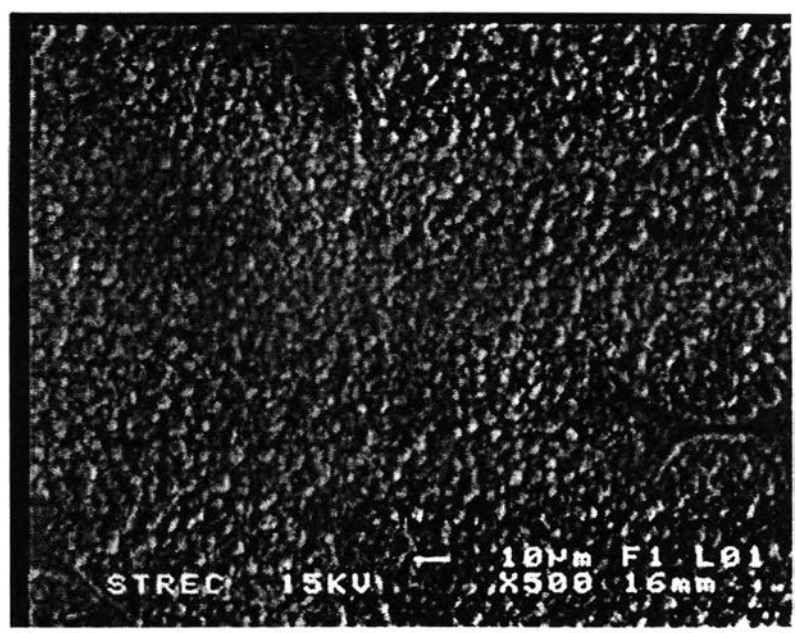


(b)

Figure 50 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (2:1); (a) Film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)

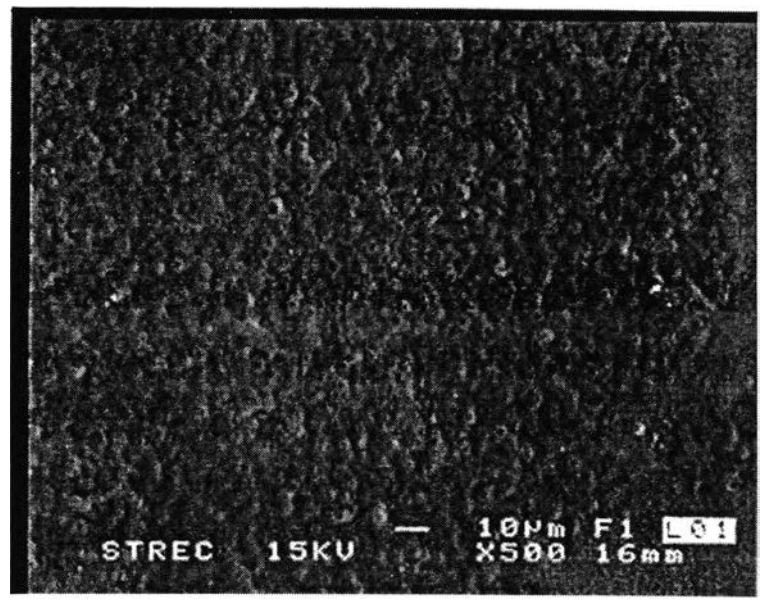


(a)

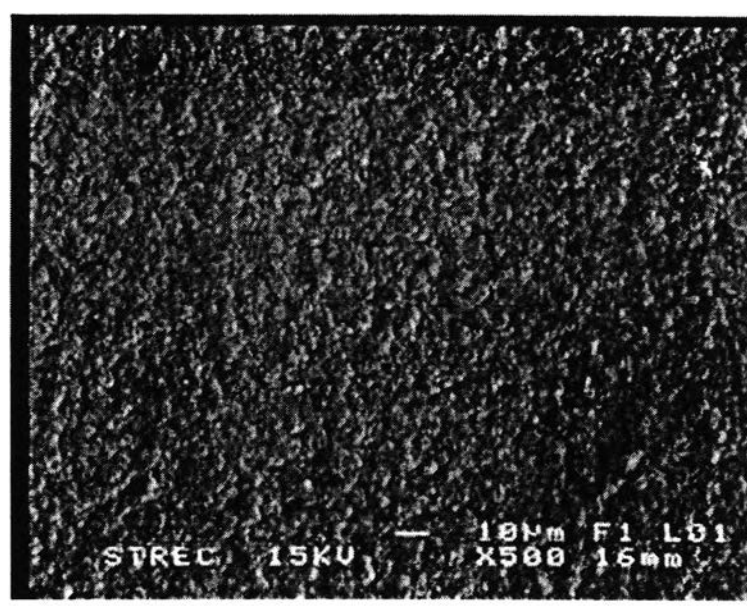


(b)

Figure 51 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (3:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)

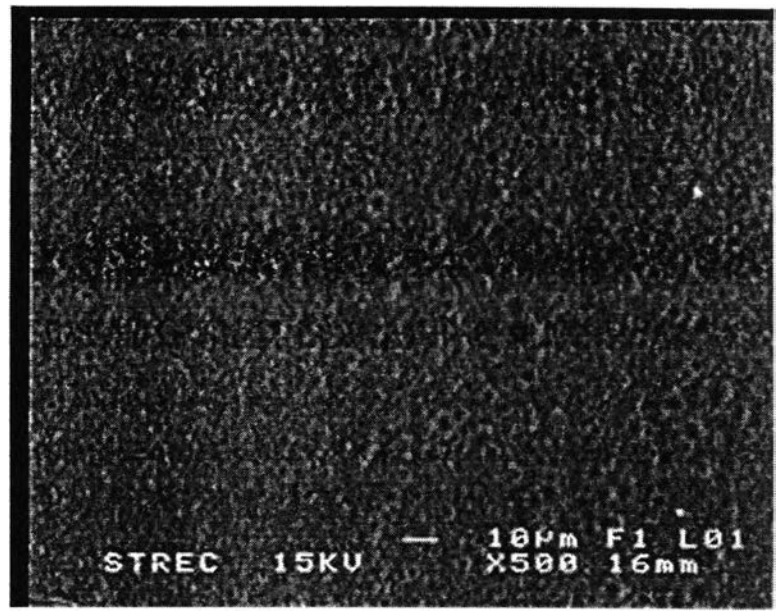


(a)

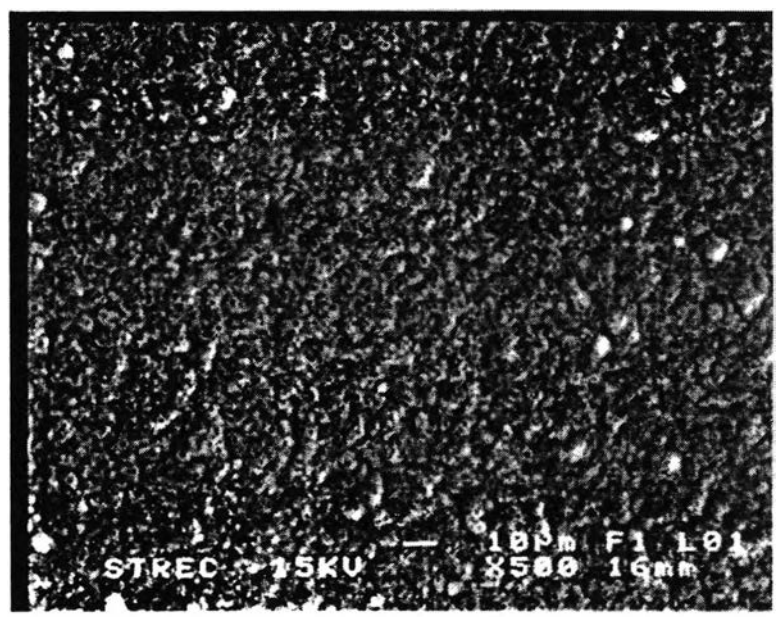


(b)

Figure 52 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (4:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)



(a)



(b)

Figure 53 The photomicrographs of surface morphology of orally fast dissolving films in formulation E3HPC (5:1); (a) film base and (b) film containing *Garcinia mangostana* extract (Magnification x 500)

2. Physicochemical characterization

2.1 Differential thermal analysis

The DSC thermograms of the ingredients in formulation consisting of HPMC 3 cps, HPC LV, acesulfame potassium, menthol, and eucalyptus oil in temperature range of -50 to 220 °C, heating rate 10 °C/minute are shown in Figures 54-55. The physical mixtures of formulation without and with extract are shown in Figures 56 and 58. The DSC thermogram of *Garcinia mangostana* extract displayed its endothermic peak at 165.75 ± 0.93 °C (Figure 30). The DSC thermogram of menthol showed endothermic peak at 46.91 °C but no observation of melting point of acesulfame potassium due to its melting point is 250 °C (Rowe, Sheskey, and Weller, 2003). However, no peaks were found in the orally fast dissolving films with and without *Garcinia mangostana* extract (Figures 57, 59 and 60). These results indicated that extract and other ingredients changed to either molecular dispersed or amorphous form.

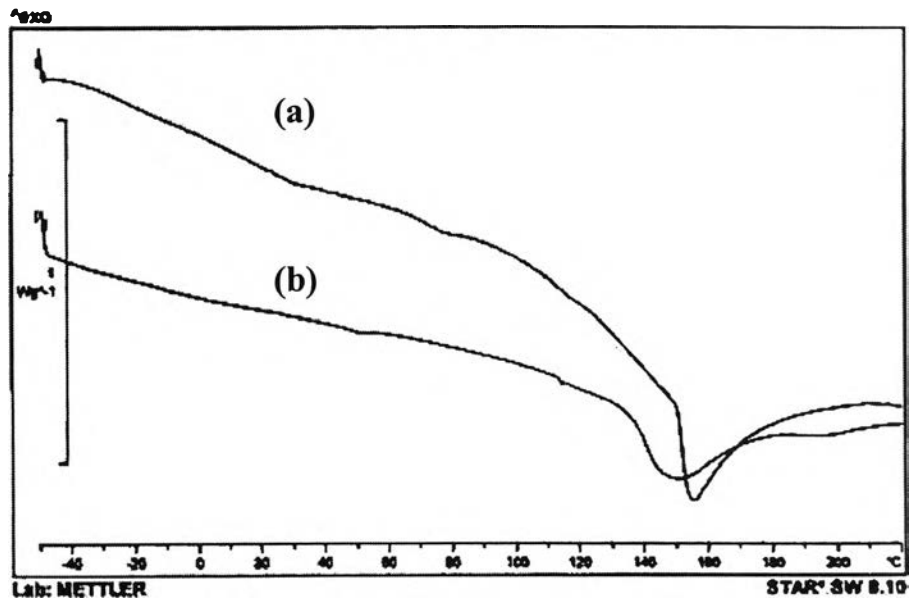


Figure 54 DSC thermograms of film former in powder form in the range of –50 to 220 °C; (a) HPMC 3 cps (sample weight 3.19 mg); (b) HPC LV (sample weight 3.15 mg)

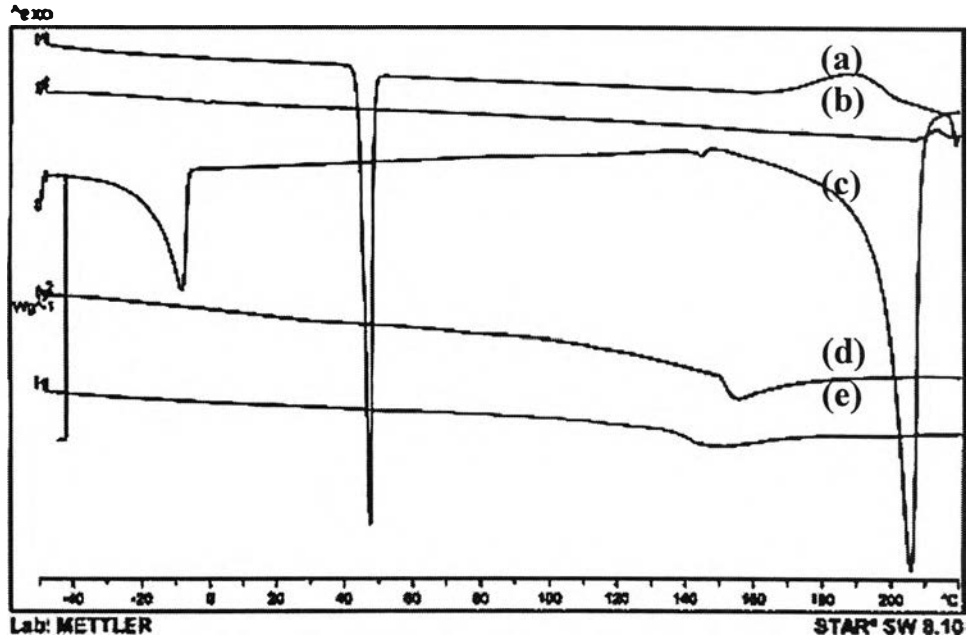


Figure 55 DSC thermograms of additive compounds in the range of –50 to 220 °C; (a) Menthol (sample weight 2.36 mg); (b) Acesulfame potassium (sample weight 3.32 mg) (c) Eucalyptus oil (sample weight 2.50 mg); (d) HPMC 3 cps (sample weight 3.19 mg); (e) HPC LV (sample weight 3.15 mg)

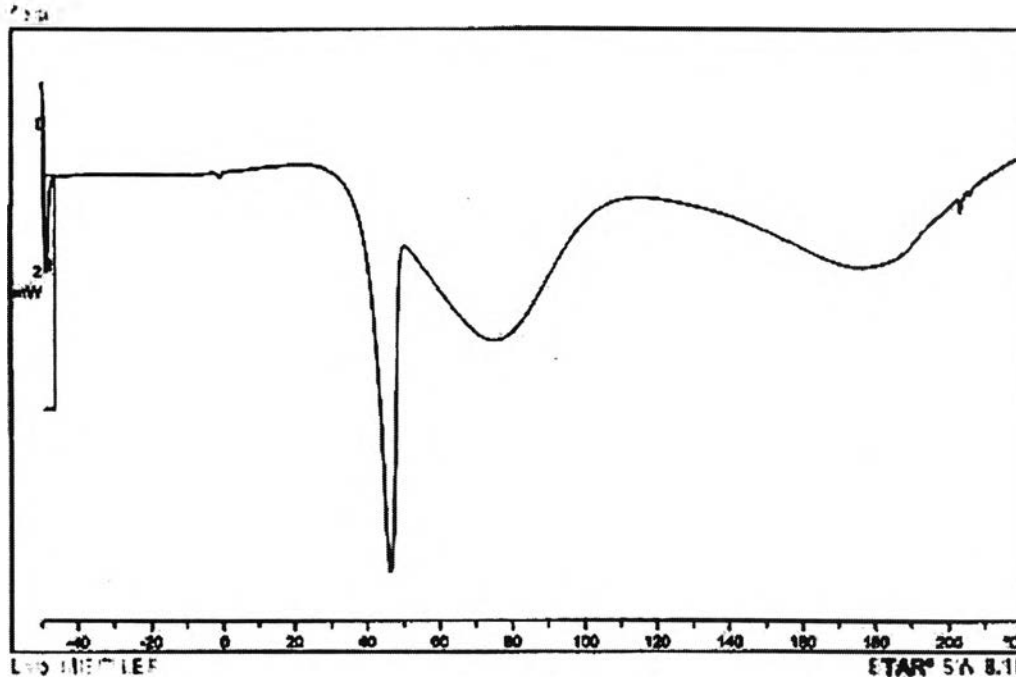


Figure 56 DSC thermograms of physical mixtures of substances in film base formula in the temperature range of –50 to 220 °C; consisted of HPMC 3 cps (sample weight 3.11 mg), HPC LV (sample weight 1.74), Acesulfame potassium (sample weight 0.64), Menthol (sample weight 1.06), and Eucalyptus oil (sample weight 0.32 mg)

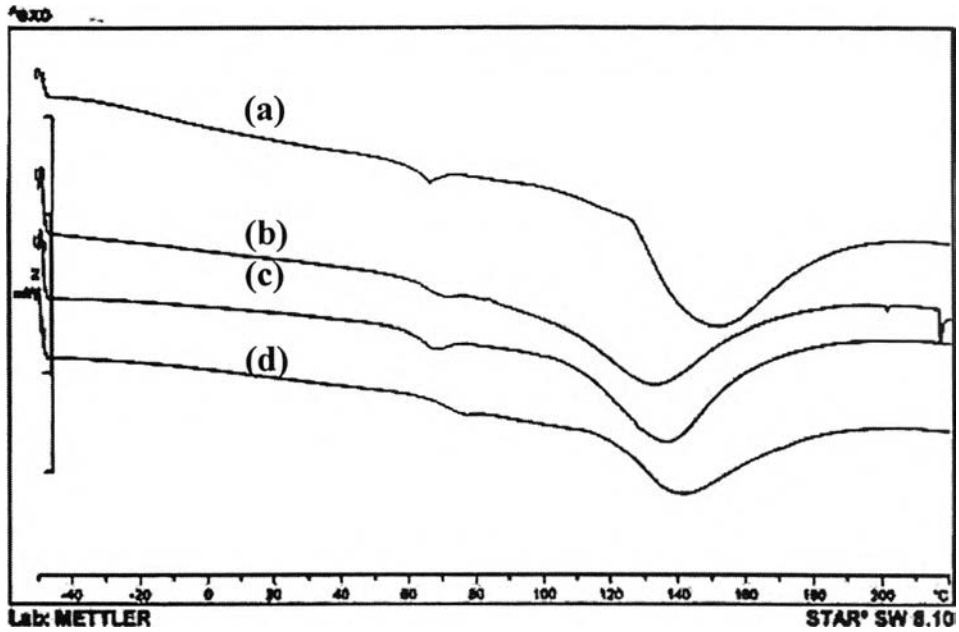


Figure 57 DSC thermograms of orally fast dissolving film bases in the temperature range of –50 to 220 °C; (a) E3HPC (2:1) (sample weight 5.07 mg); (b) E3HPC (3:1) (sample weight 4.70 mg); (c) E3HPC (4:1) (sample weight 4.96 mg); (d) E3HPC (5:1) (sample weight 4.93 mg)

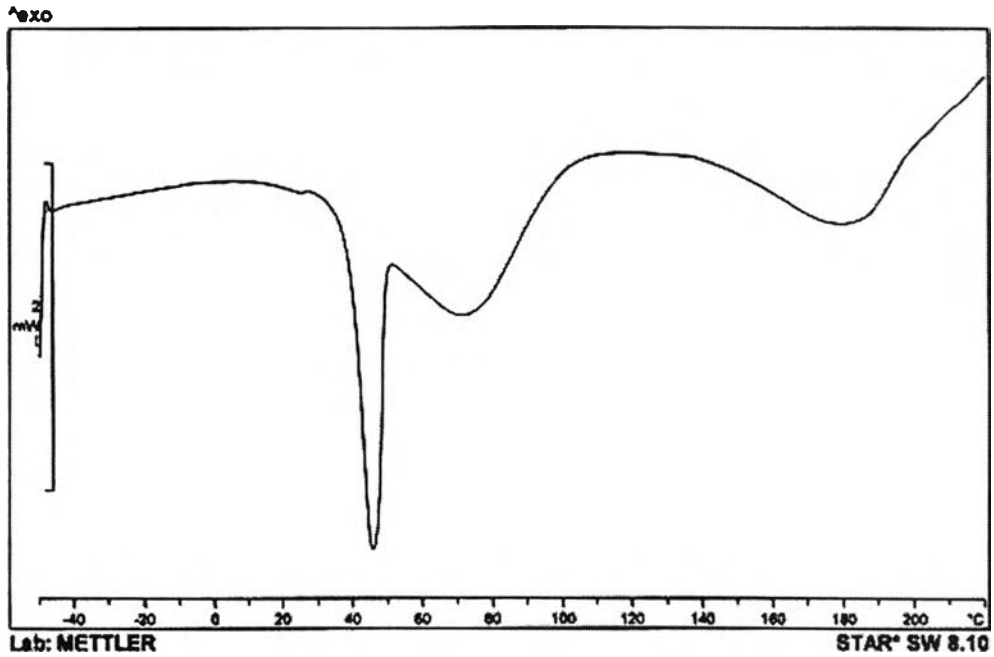


Figure 58 DSC thermograms of physical mixtures of substances in film containing extract formula in the temperature range of –50 to 220 °C; consisted of HPMC 3 cps (sample weight 2.73 mg), HPC LV (sample weight 1.16), Acesulfame potassium (sample weight 1.25), Menthol (sample weight 1.34), Eucalyptus oil (sample weight 0.44 mg), and *Garcinia mangostana* extract (sample weight 0.29 mg)

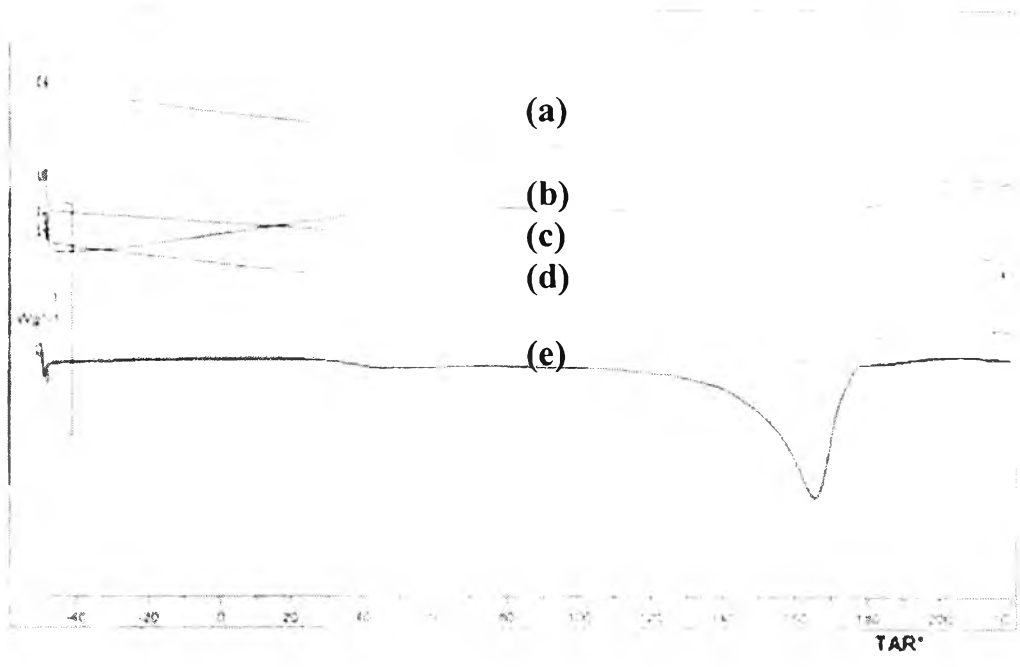


Figure 59 DSC thermograms of films in formulation of E3HPC (2:1) and (3:1) in the temperature range of –50 to 220 °C; (a) E3HPC (2:1) film base (sample weight 5.07 mg); (b) E3HPC (2:1) containing *Garcinia mangostana* extract (sample weight 4.60 mg); (c) E3HPC (3:1) film base (sample weight 4.70 mg); (d) E3HPC (3:1) containing *Garcinia mangostana* extract (sample weight 5.00 mg); (e) *Garcinia mangostana* extract (sample weight 3.27 mg)

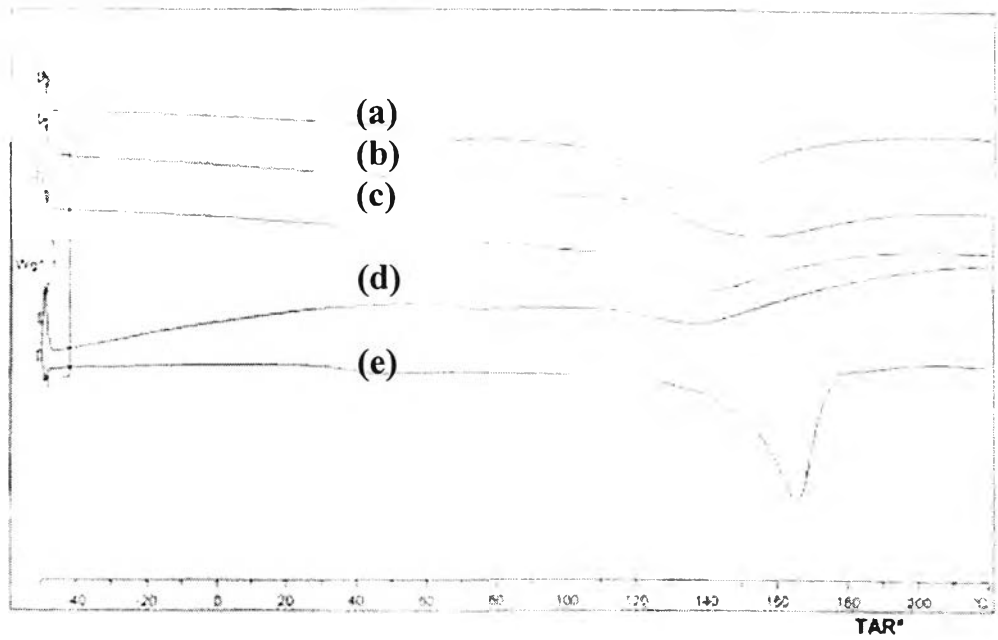


Figure 60 DSC thermograms of films in formulation of E3HPC (4:1) and (5:1) in the temperature range of –50 to 220 °C; (a) E3HPC (4:1) film base (sample weight 4.96 mg); (b) E3HPC (4:1) containing *Garcinia mangostana* extract (sample weight 5.32 mg); (c) E3HPC (5:1) film base (sample weight 4.93 mg); (d) E3HPC (5:1) containing *Garcinia mangostana* extract (sample weight 5.30 mg); (e) *Garcinia mangostana* extract (sample weight 3.27 mg)

3. Weight variation of orally fast dissolving films containing *Garcinia mangostana* extract

The results of weight variation are presented in Appendix C. It shows in Table 30 that the weight of all test films was within the limit as 21.25±0.52 to 24.39±0.56 mg.

Table 30 Weight variation of orally fast dissolving films containing *Garcinia mangostana* extract

Formulas	Average weight ± SD (mg)	%CV
E3HPC (2:1)	21.25 ± 0.52	2.43
E3HPC (3:1)	24.39 ± 0.56	2.31
E3HPC (4:1)	23.92 ± 0.82	3.43
E3HPC (5:1)	24.13 ± 0.65	2.71

4. Thickness of orally fast dissolving films containing *Garcinia mangostana* extract

The results of thickness of films are presented in Appendix C. It shows in Table 31 that the thickness of all test films was within the limit as 35.4±0.48 to 38.3±0.88 μm.

Table 31 The average thickness of the orally fast dissolving films containing *Garcinia mangostana* extract (n=5 each sample was measured at 5 locations).

Formulas	Average thickness ± SD. (μm)					Mean ± SD
	No. 1	No. 2	No. 3	No. 4	No. 5	
E3HPC (2:1)	35.2±1.10	35.6±1.52	36.2±1.30	35.0±0.71	35.2±0.45	35.4±0.48
E3HPC (3:1)	36.4±1.34	36.8±0.45	35.4±0.89	37.6±1.14	36.6±1.14	36.6±0.79
E3HPC (4:1)	36.8±1.10	39.0±1.00	38.6±0.89	38.8±1.10	38.2±1.10	38.3±0.88
E3HPC (5:1)	35.6±0.55	35.0±1.22	36.6±1.34	35.4±0.55	35.4±1.14	35.6±0.60

5. Mechanical properties of orally fast dissolving films containing *Garcinia mangostana* extract

Mechanical properties of films showed in values of tensile strength, percentage of elongation at break, Young's modulus and work of failure (Table 32). From the results, it was found that the ultimate tensile strength and Young's modulus values of fast dissolving films containing *Garcinia mangostana* extract were highly than of film bases in all formulations. It indicated that the films containing *Garcinia mangostana* extract were rigid and harder than film bases. While percent elongation and work of failure values of film bases were more than of film containing extract. These results suggested that film bases were tougher than its film containing extract (Figure 61-64).

Table 32 Mechanical properties of orally fast dissolving films containing *Garcinia mangostana* extract (n=5).

Formulas	Ultimate tensile strength ±SD (MPa)	% Strain at point of break ±SD (%)	Young's modulus ±SD (MPa)	Work of failure ±SD(mJ)
E3HPC (2:1)	44.02 ± 1.01	13.36 ± 0.55	429.24 ± 13.58	1.04 ± 0.08
E3HPC (3:1)	49.10 ± 0.5	13.38 ± 0.34	465.72 ± 13.36	1.21 ± 0.08
E3HPC (4:1)	52.00 ± 2.24	13.62 ± 0.79	477.08 ± 14.02	1.22 ± 0.11
E3HPC (5:1)	47.44 ± 1.18	13.58 ± 0.79	443.72 ± 21.30	1.11 ± 0.09

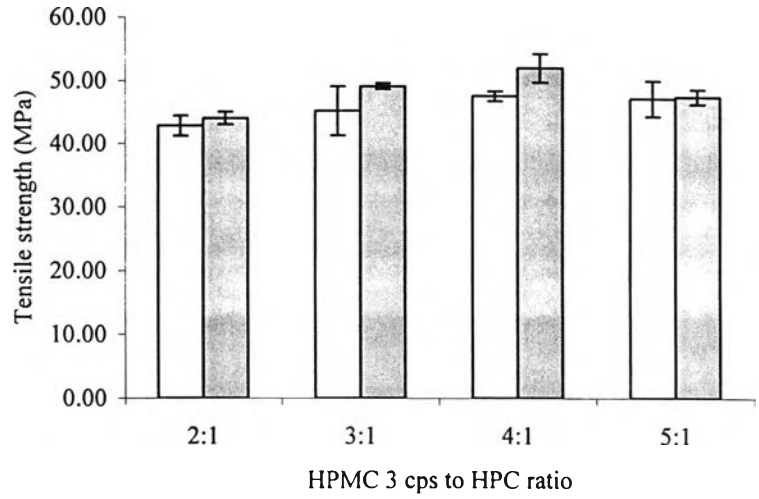


Figure 61 Tensile strength of orally fast dissolving films; □ film bases; ▨ film containing *Garcinia mangostana* extract

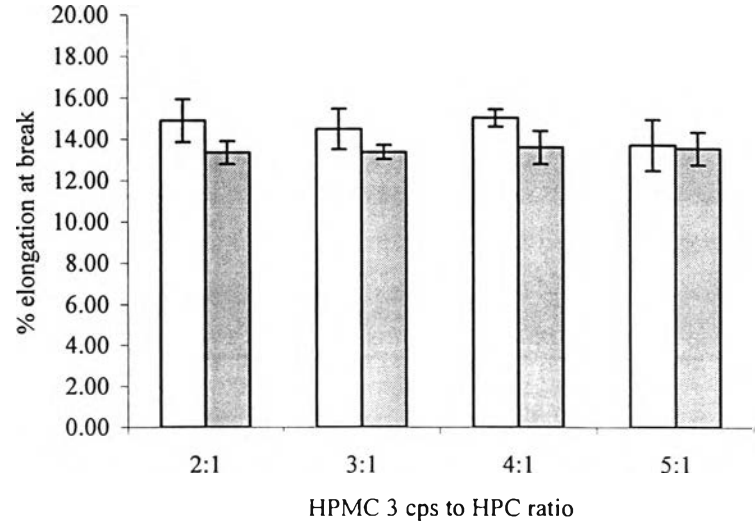


Figure 62 Percentage of Elongation at break of orally fast dissolving films; □ film bases; □ film containing *Garcinia mangostana* extract

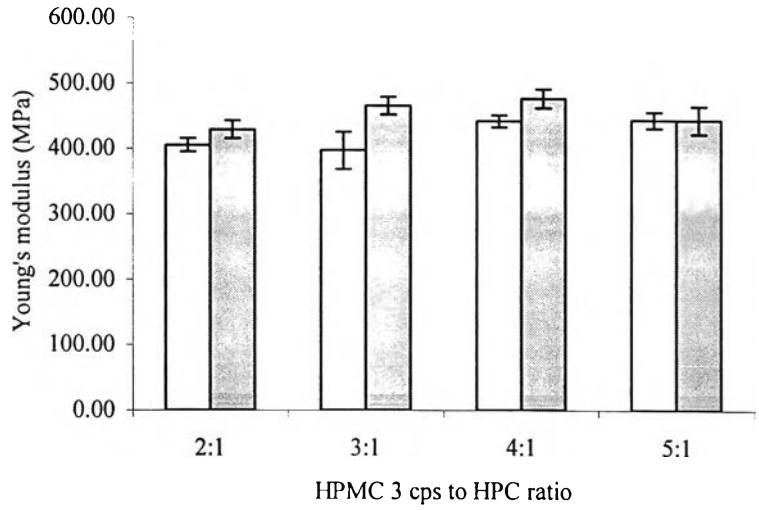


Figure 63 Young's modulus of orally fast dissolving films; ☐ film bases; ▨ film containing *Garcinia mangostana* extract

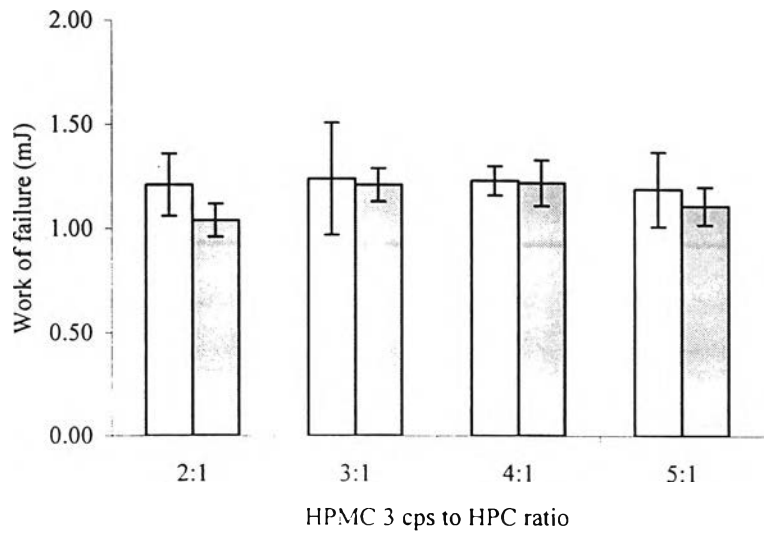


Figure 64 Work of failure of orally fast dissolving films; ☐ film bases; ☐ film containing *Garcinia mangostana* extract

6. *In vitro* dissolution time of orally fast dissolving films containing *Garcinia mangostana* extract

The method for observing the dissolution time of films in this study was modified from Xu et al.(2002). Unlike the film bases, when the film containing the extract floated on the surface of isotonic phosphate buffer pH 6.2 at temperature 37 ^{o}C, it disintegrated into many small pieces and gave yellow and cloudy solution. This observation may be due to the extract has poor solubility in this buffer. Therefore, the dissolution time of the film containing extract in this study was the time that film completely disintegrated into small pieces. From the result of dissolution time of films, it was found that the films containing extract disintegrated slower than film bases in all formulations. It may be due to hydrophobic characteristic of the extract interfered hydrophilic group in polymers to bind with water molecule. Only E3HPC (2:1) films containing extract were not significantly different in dissolution time from commercial product strips A (p>0.05).

Table 33 Dissolution time of orally fast dissolving films containing *Garcinia mangostana* extract (n=5).

Formulas	Dissolution time (sec)					Mean ± SD	% CV
	No. 1	No. 2	No. 3	No. 4	No. 5		
E3HPC (2:1)	102	128	140	132	114	123.2 ± 15.14	12.29
E3HPC (3:1)	460	432	485	490	507	474.8 ± 29.25	6.16
E3HPC (4:1)	367	435	384	402	430	403.6 + 29.19	7.23
E3HPC (5:1)	209	222	160	180	160	186.2 ± 28.34	15.22

7. Content uniformity of orally fast dissolving films containing *Garcinia mangostana* extract

The dosage-unit uniformity was determined by assay of 10 individual units (2 x 3 cm^2) using the HPLC method previously described (Appendix C, Table C4). The percentage content of mangostin was calculated based on mangostin content at 181.61 μg/strip. Table 34 informs that the drug content in the 10 individual units of prepared orally fast dissolving films lied within the range of 85.0-115.0% of labeled amount with the relative standard deviation (RSD) was less than 6.0%. Therefore, these fast

dissolving films containing *Garcinia mangostana* extract met the requirement of content uniformity (USP 26/NF 21, 2003).

Table 34 The average of the percent content of mangostin in the prepared fast dissolving films (n=10).

Formulas	%content ± SD	% Labeled amount ± SD	%RSD
E3HPC (2:1)	170.68 ± 0.43	93.98 ± 2.37	2.52
E3HPC (3:1)	193.13 ± 0.75	106.34 ± 4.15	3.90
E3HPC (4:1)	179.50 ± 0.59	98.84 ± 3.26	3.30
E3HPC (5:1)	184.80 ± 0.58	101.75 ± 3.19	3.14

8. *In vitro* dissolution study of orally fast dissolving films containing *Garcinia mangostana* extract

8.1 Selection of dissolution medium

Due to the pH of saliva fluid was reported in the range from 5.8 to 7.4 (Rathbone, 1996), therefore, isotonic phosphate buffer pH 6.2 was used in this study. However, mangostin was poorly soluble in this medium, sodium lauryl sulfate was added into the medium to enhance drug solubility. In this study, sodium lauryl sulfate ranging form 0-1.0% was added into the dissolution medium and the solubility of mangostin was determined. The results showed that the solubility of mangostin was increased as a function of sodium lauryl sulfate concentration (Table 35). The solubility of mangostin in isotonic phosphate buffer pH 6.2 was 0.5606 μg/ml and in the present of 1.0% sodium lauryl sulfate, the solubility was increased to 952.314 μg/ml, which was the lowest concentration of sodium lauryl sulfate that was able to maintain the sink condition in the dissolution study.

Table 35 Solubility of mangostin in isotonic phosphate buffer pH 6.2 without and with various concentrations of sodium lauryl sulfate (SLS)

Medium	Solubility (μg/ml)
isotonic phosphate buffer pH 6.2	0.5606 ± 0.095
isotonic phosphate buffer pH 6.2 + 0.1% SLS	73.339 ± 2.78
isotonic phosphate buffer pH 6.2 + 0.2% SLS	137.535 ± 3.64
isotonic phosphate buffer pH 6.2 + 0.3% SLS	145.247 ± 3.89
isotonic phosphate buffer pH 6.2 + 0.4% SLS	171.908 ± 9.52
isotonic phosphate buffer pH 6.2 + 0.5% SLS	241.123 ± 7.53
isotonic phosphate buffer pH 6.2 + 0.75% SLS	449.074 ± 3.11
isotonic phosphate buffer pH 6.2 + 1.0% SLS	952.314 ±15.57

8.2 *In vitro* dissolution study

In vitro drug release study are frequently used to gain information about the release profiles of active ingredients in the formulation development. In the present work, the release study of all formulations were carried out using a modified dissolution apparatus and isotonic phosphate buffer pH 6.2 with 1.0% sodium lauryl sulfate as a dissolution medium. The amounts of drug release were analyzed using HPLC. The validation results were shown in the previous part.

The dissolution profiles were plotted between the cumulative amounts of mangostin released from orally fast dissolving films in different ratios of HPMC 3 cps and HPC LV combination versus time as shown in Figures 65-68. The dissolution data are presented in Appendix C.

From the result, it was found that mangostin rapidly released into the dissolution medium. About 80% of labeled amount was released within 3 minutes for E3HPC (2:1) and E3HPC (5:1) and within 5 and 7 minutes for E3HPC (4:1) and E3HPC (3:1), respectively. These findings may support the observation from scanning electron micrographs as shown in Figure 50(b)-53(b) that the fast dissolving films containing *Garcinia mangostana* extract with combination of HPMC 3 cps and HPC LV as film formers provided porous and spongy-like surface. Therefore, the high porosity of these films might affect the fast release rate.

In addition, the film formers, both HPMC 3 cps and HPC LV are hydrophilic polymers. They have ability to absorb water, thereby promoting the dissolution. Moreover, the hydrophilic polymers would leach out and, hence, create more pores and channels for the drug to diffuse out of the patches (Wong, Yuen, and Peh, 1999).

Drug molecules are released from hydrocolloid matrices by the following 3 mechanisms:

1. Water induced relaxation of the polymer matrix
2. Erosion of the polymer gel layer surrounding the matrix dosage form
3. Diffusion of drug molecules through the swollen gel layer

The relative significance of these three mechanisms depends on the properties of the drugs and polymers. Drug release mechanism can generally be expressed by the following equation:

$$Q = kt^n$$

In this equation, n is the diffusional exponent for the drug release, k is the dissolution rate constant giving a measure of the velocity of drug release and Q is the cumulative amount of drug release in a certain time interval (t).

The cellulose ether derivatives swell infinitely by absorbing water and concomitantly dissolve from the surface of the system. When polymer swelled the void spaces were increased with the polymer unfolded and the coil hydrated. The drug release from the systems occurs by diffusion of the molecules across these voids of the polymer matrix and also by the polymer from the surface. However, the viscosity of the gel determines the mechanism of the drug release from these systems. The high viscosity in the pores serves to retard the diffusion of the drug at the early stages of release. While hydration of the low molecular weight polymer occurred rapidly. After this period, significant erosion of the surface due to the convective movement of the solvent past the film was observed. The eroded particles exposed greater surface area for drug release, resulting in a progressive increase in release rate. Therefore, the

release of drug from the low molecular weight and less viscous polymers (shorter chain length) is controlled by the dissolution of the polymer and follows zero order kinetics and the mechanism involved is erosion or relaxation controlled (n=1) (Lapidus and Lordi, 1968; Korsmeyer et al., 1983; Repka et al., 2005).

$$\text{Zero-order equation} : \quad Q_t = Q_0 + k_0 t$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and k_0 is the zero order release constant.

Mockel and Lippold (1993) reported that drug release slowed down with increasing degree of viscosity of the gel forming polymer. The exponent n decreased from 1 to approx. 0.5 with increasing solution viscosity. The release from low molecular weight polymers with a lower degree of viscosity proceeded according to zero-order kinetics, whereas a higher viscosity resulted in drug release showing an approximately square root of time dependency. For the polymers with the same chemical structure, the dissolution rate decreased with rising molecular weight which can be accounted for by the increase in the thickness of the swollen layer.

This agreed with Taweekunthum (2001) who reported that the drugs would be trapped into more viscous hydrated films more than in lower viscous hydrated films resulting in reduction in drug release. Since the polymer systems utilized in this study were low viscous, which eroded easily in a few minutes so films exhibited an initial burst effect that would be advantageous for rapid onset of action.

In this study, the zero-order kinetics was applicable for the fast dissolving films containing *Garcinia mangostana* extract, as amount released versus time when plotted with the obtained dissolution data at initial part were linear. Although, the fastest and highest of mangostin release rate was observed in formulation E3HPC (5:1), the analysis of data showed that there was no statistically significant difference at initial stage (1 and 3 minutes) and overall of percentage of drug release in all formulations (p>0.05).

Table 36 Zero order kinetic parameters of mangostin release from orally fast dissolving films containing *Garcinia mangostana* extract

Formulations	Zero-order plot	
	k_0	R^2
E3HPC (2:1)	11.5440	0.8656
E3HPC (3:1)	7.5746	0.8985
E3HPC (4:1)	8.8800	0.9220
E3HPC (5:1)	14.8090	0.8786

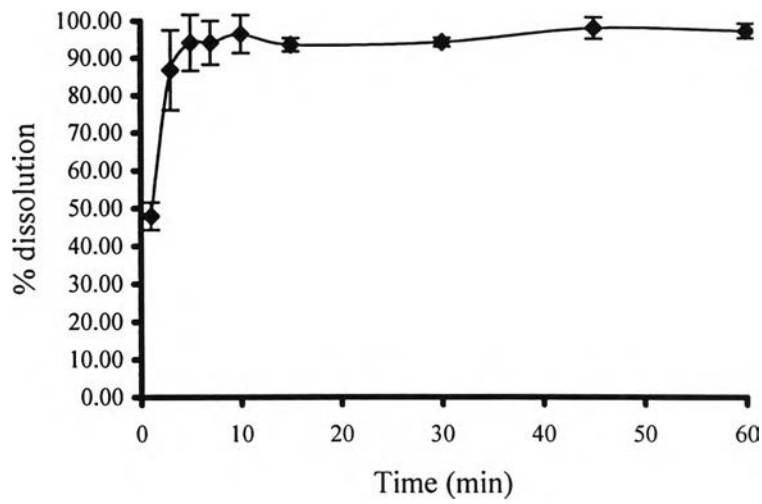


Figure 65 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (2:1)

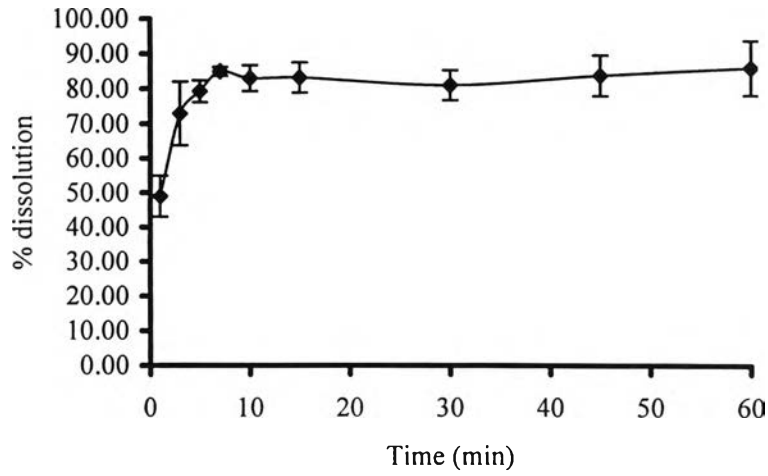


Figure 66 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (3:1)

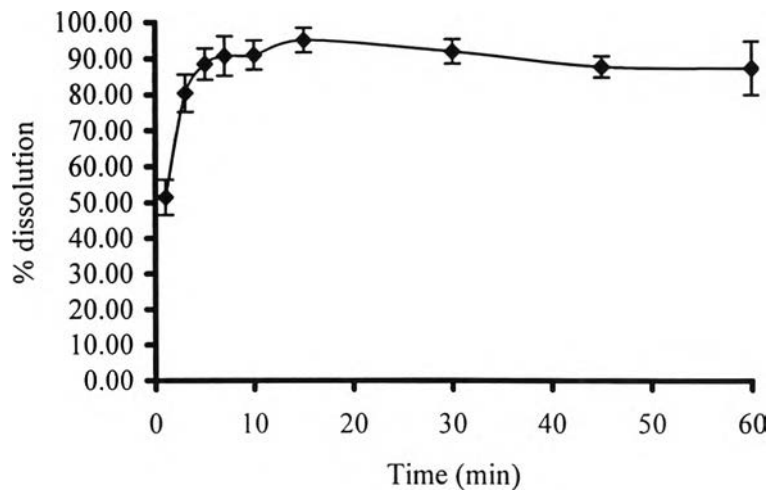


Figure 67 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (4:1)

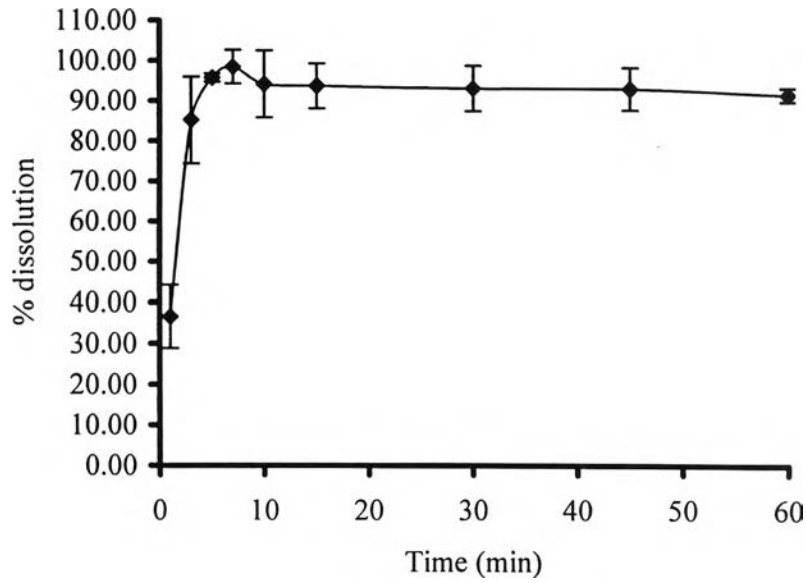


Figure 68 Dissolution profile of mangostin from orally fast dissolving film in formulation E3HPC (5:1)

H. Antimicrobial Activity of Orally Fast Dissolving Films Containing *Garcinia mangostana* extract

All formulations of orally fast dissolving films containing *Garcinia mangostana* extract were tested against microorganisms commonly found in oro-dental infections namely *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC $KPSK_2$ using agar diffusion method.

1. Antimicrobial activity of dried films

The dried fast dissolving films containing *Garcinia mangostana* extract with diameters of 5.50 mm were place on Mueller Hinton agar plates that were swabbed with inoculum in concentration 10^8 cells/ml. It was found that films disintegrated and spread around due to the films absorbed water in agar medium, therefore the clear zone was observed as shown in Figures 69-73 and Table 38. This result suggested that the film containing extract had antimicrobial activity against both test bacteria.

However, the inhibition zone that exerted from films containing *Garcinia mangostana* extract was less than from film containing cetylpyridinium chloride and chlorhexidine diacetate in the same concentration of extract and same formulation of film. These results may be due to the solubility of cetylpyridinium chloride and chlorhexidine diacetate in water were more than of extract. It was possible that cetylpyridinium chloride and chlorhexidine diacetate could more diffuse in agar medium than extract. The water solubility of cetylpyridinium chloride and chlorhexidine diacetate are 1 in 20 and 1 in 55 of water (Rowe, Sheskey, and Weller, 2003), whereas the solubility of extract is 0.028 ± 0.0045 mg/ml (Hiranras, 2001). Additionally, cetylpyridinium chloride and chlorhexidine diacetate have much lower MIC value than that of extract.

From observation of commercial product strips A films, it was found that the area of films was less dense than that around on agar medium but it was not completely clear zone as shown in Figure 69(a). This might reveal that commercial product strips A had no antibacterial activity in this test.

Due to the flavoring agents of all formulas were menthol and eucalyptus oil, which has antimicrobial effect, especially menthol can inhibit both *Staphylococcus aureus* and showed weak activity against *Streptococcus mutans* at MIC value of 400 μg/ml (Iscan et al., 2002; Trivedi and Hotchandani, 2004). Therefore, the polymeric films (only film formers without any additives) and film bases (had additives) of all ratios of formulas were prepared and also test the antimicrobial activity for the control study. It was found that the polymeric film did not show any clear zone but the film base exhibited the reduction of growth of both bacteria due to dense of bacteria under film was less than around. This considerable experimental evidence suggested that was antimicrobial effect of additives in formulation such as menthol and eucalyptus oil.

To clarify the amount of extract in formulation that may affect *in vitro* antimicrobial activity test, the film containing extract in 2, 4, and 6 times of one of standard formula E3HPC (5:1) were prepared, but film that had 6 times of amount of extract was very brittle and easy to break. The analysis of antimicrobial activity

results showed that there was no statistically significant difference between amount of mangostin in E3HPC (5:1) film (p>0.05).

Table 37 Antimicrobial activity of fast dissolving film using agar diffusion method (n=3)

Film formulations	Inhibition zone (mm±SD)	
	Staphylococcus aureus ATCC 25923	*Streptococcus mutans* $KPSK_2$
Fast dissolving film containing *Garcinia mangostana* extract		
E3HPC (2:1)	8.23±1.36	6.67±0.29
E3HPC (3:1)	7.13±0.78	7.20±0.58
E3HPC (4:1)	7.55±0.60	6.17±0.45
E3HPC (5:1)	8.35±0.26	5.88±0.30
E3HPC (5:1) extract x 2	7.02±0.63	5.85±0.33
E3HPC (5:1) extract x 4	6.82±0.76	5.72±0.34
E3HPC (5:1) extract x 6	7.52±0.60	5.85±0.25
Commercial product strips A	0.00	0.00
Fast dissolving film containing cetylpyridinium chloride		
E3HPC (2:1)	35.93±6.82	32.65±2.41
E3HPC (3:1)	37.65±3.31	36.12±2.83
E3HPC (4:1)	35.92±1.26	30.25±7.97
E3HPC (5:1)	41.03±1.93	29.55±2.86
Fast dissolving film containing chlorhexidine diacetate		
E3HPC (2:1)	24.40±1.19	27.97±0.65
E3HPC (3:1)	24.27±1.10	26.83±1.50
E3HPC (4:1)	24.47±3.07	29.08±0.38
E3HPC (5:1)	24.43±2.18	27.67±1.22

* Average of diameters of film sample were 5.50 mm.

2. Antimicrobial activity of solution of fast dissolving film

The solution of fast dissolving film consisted of a fast dissolving film completely dissolve or disintegtated in 2 ml of isotonic phosphate buffer pH 6.2 as simulated saliva. The film solution was dropped in sterile cup that place on Mueller Hinton agar swabbed with 10^8 cells/ml of inoculum. The results found that no inhibition zone was observed in isotonic phosphate buffer pH 6.2, polymeric film and film base as negative control (Figures 74-75). Commercial product strips A film solution also showed no inhibition zone, while commercial mouth wash product B, consisted of high antiseptic volatile oil, and , commercial mouth wash product C consisted of 0.12% w/v of chlorhexidine gluconate, exhibited antimicrobial activity against both types of bacteria (Table 38).

As the same result from dried films, the solution of fast dissolving film containing *Garcinia mangostana* extract had higher antimicrobial activity against *Staphylococcus aureus* ATCC 25923 than *Streptococcus mutans* ATCC $KPSK_2$. This was a inversely result that from in the study of cetylpyridinium chloride and chlorhexidine diacetate.

Table 38 Antimicrobial activity of solution of fast dissolving film using agar diffusion method (n=3)

Film formulations	Inhibition zone (mm±SD)	
	Staphylococcus aureus ATCC 25923	*Streptococcus mutans* $KPSK_2$
Fast dissolving film containing *Garcinia mangostana* extract		
E3HPC (2:1)	8.43±0.58	6.82±0.33
E3HPC (3:1)	8.58±1.06	6.87±0.39
E3HPC (4:1)	9.13±0.13	8.03±2.30
E3HPC (5:1)	10.47±1.09	7.48±1.42
E3HPC (5:1) extract x 2	12.13±2.66	7.78±1.56
E3HPC (5:1) extract x 4	13.55±1.98	8.27±2.01
E3HPC (5:1) extract x 6	12.33±0.19	9.55±3.18
Commercial product strips A	0.00	0.00
Commercial mouth wash product B	13.58±4.68	6.06±0.04
Commercial mouth wash product C	18.48±0.65	24.45±1.95
Fast dissolving film containing cetylpyridinium chloride		
E3HPC (2:1)	10.55±0.78	15.47±1.00
E3HPC (3:1)	12.72±2.08	13.39±0.88
E3HPC (4:1)	15.02±4.32	16.97±0.98
E3HPC (5:1)	14.52±3.86	17.15±0.58
Fast dissolving film containing chlorhexidine diacetate		
E3HPC (2:1)	18.70±0.40	20.90±2.68
E3HPC (3:1)	18.42±0.78	23.18±1.40
E3HPC (4:1)	17.90±0.17	24.17±1.07
E3HPC (5:1)	16.42±0.43	22.10±1.43

* Average diameters of sterile cups were 6.0 mm.

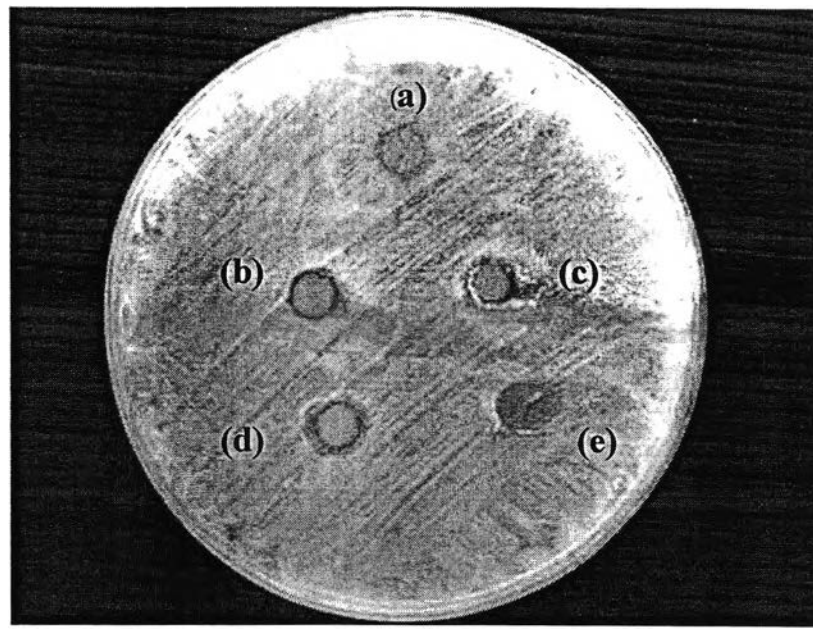


Figure 69 Agar diffusion test of fast dissolving film against *Staphylococcus aureus* ATCC 25923; (a) Commercial product strips A; (b) to (e) film containing extract in amount of 2, 4, 6 and 1 times, respectively

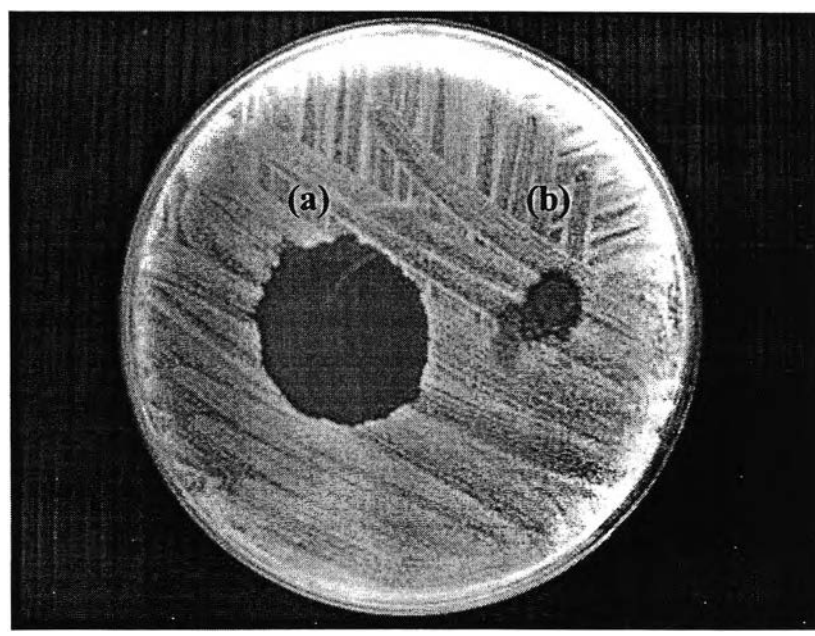


Figure 70 Agar diffusion test of fast dissolving film against *Staphylococcus aureus* ATCC 25923; (a) Chlorhexidine diacetate film; (b) film containing *Garcinia mangostana* extract

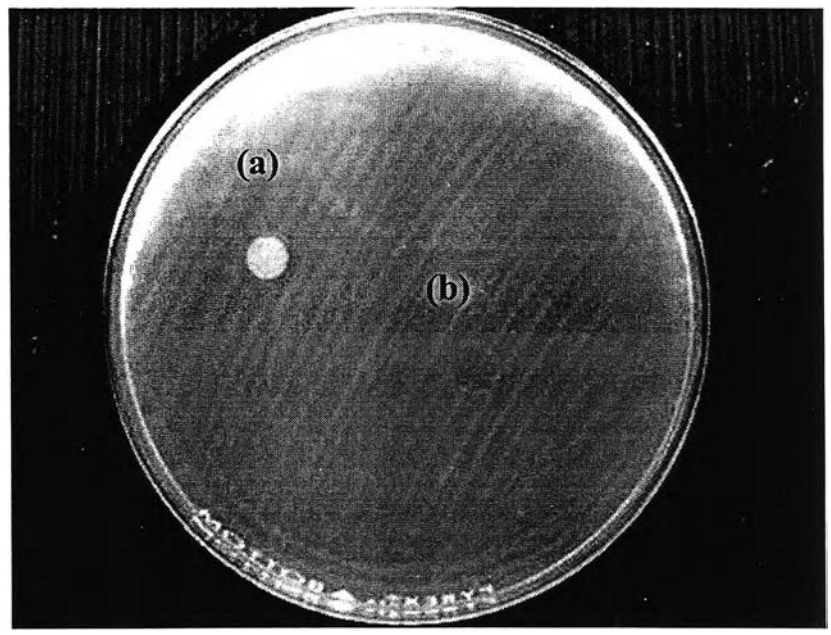


Figure 71 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) Commercial product strips A

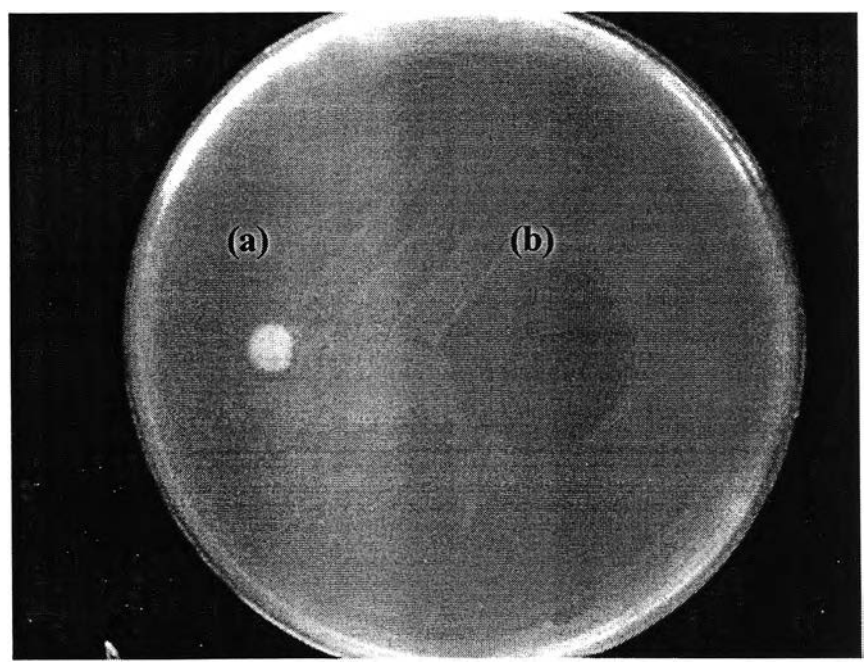


Figure 72 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) film with chlorhexidine diacetate

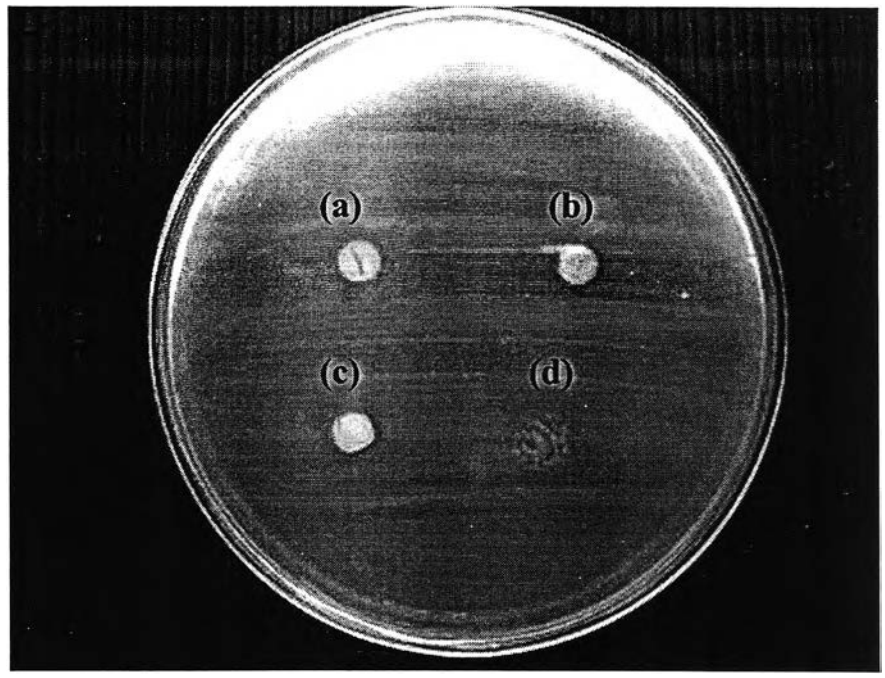


Figure 73 Agar diffusion test of fast dissolving film against *Streptococcus mutans* ATCC $KPSK_2$; (a) to (d) film containing extract in amount of 2, 4, 6 and 1 times, respectively

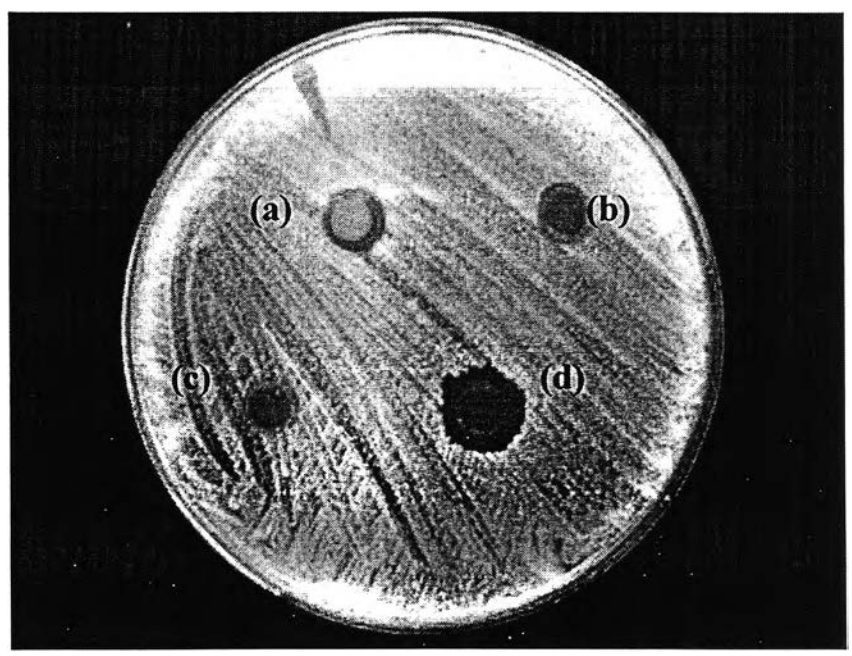


Figure 74 Agar diffusion test of fast dissolving film solution against *Staphylococcus aureus* ATCC 25923; (a) film with extract; (b) film base; (c) polymeric film without additives; (d) film containing cetylpyridinium chloride

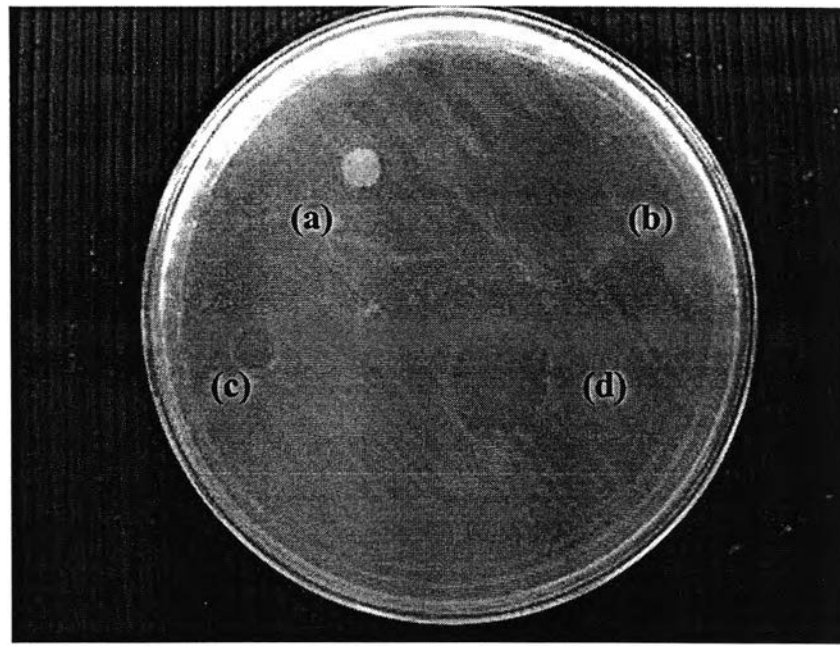


Figure 75 Agar diffusion test of fast dissolving film solution against *Streptococcus mutans* ATCC $KPSK_2$; (a) film with extract; (b) film base; (c) polymeric film without additives; (d) film containing cetylpyridinium chloride

I. Stability Study of Orally Fast Dissolving Films Containing *Garcinia mangostana* Extract

The stability study of *Garcinia mangostana* extract in orally fast dissolving films was performed by triplicate samples of four formulations. It individually packed in glass vials, which tightly sealed with rubber closures and aluminium caps and stored at 40 °C, 75 %RH for three months. At the initial time, first, second and third month, the films were sampled and assayed for the remaining mangostin content. The physicochemical properties and the amount of mangostin of orally fast dissolving films in stability test were determined using differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC), respectively.

1. Physicochemical stability study

The orally fast dissolving films containing *Garcinia mangostana* extract change in the physicochemical properties of the film was observed from DSC thermograms.

As the results of the DSC thermograms of all formulations, no change in DSC thermograms between three months of stability study (at the initial time, first, second and third month) was observed. These results indicated that all formulations did not change in physicochemical properties after the stress condition for 3 months (Figure 76-79).

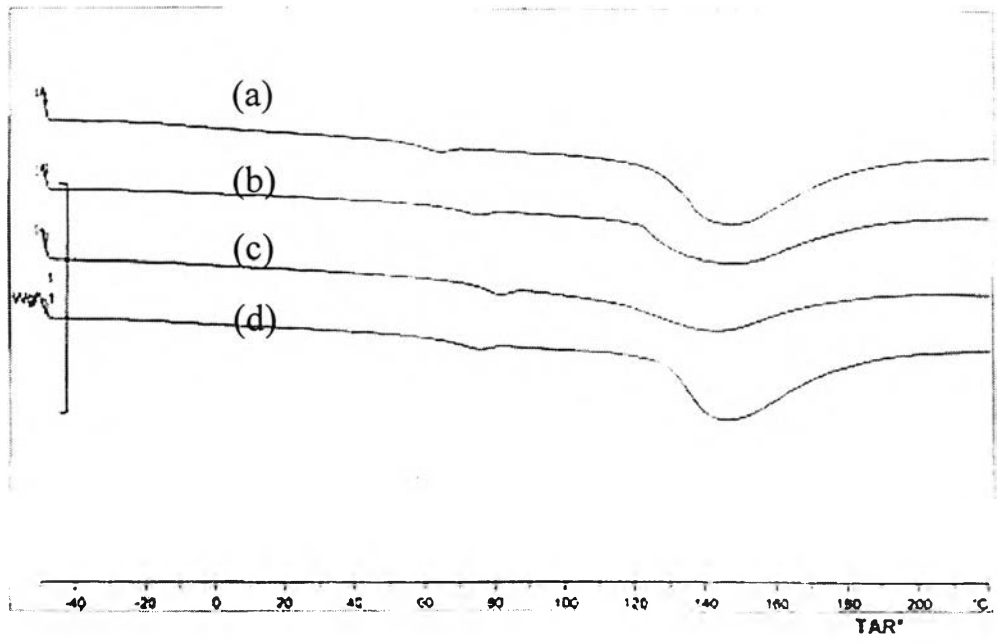


Figure 76 DSC thermograms of films in formulation E3HPC (2:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 4.96 mg); (b) at first month (sample weight 4.89 mg); (c) at second month (sample weight 5.04 mg); (d) at third month (sample weight 4.77 mg).

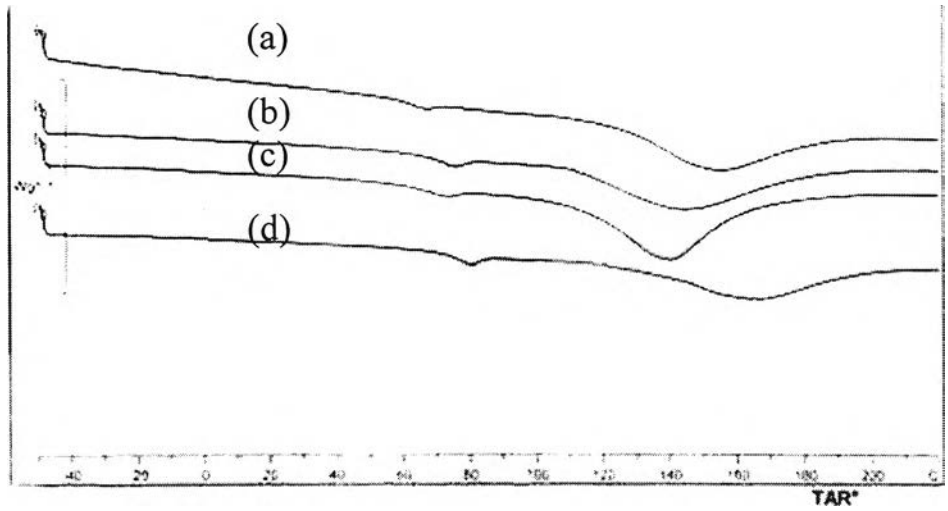


Figure 77 DSC thermograms of films in formulation E3HPC (3:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.00 mg); (b) at first month (sample weight 4.65 mg); (c) at second month (sample weight 4.98 mg); (d) at third month (sample weight 4.92 mg).

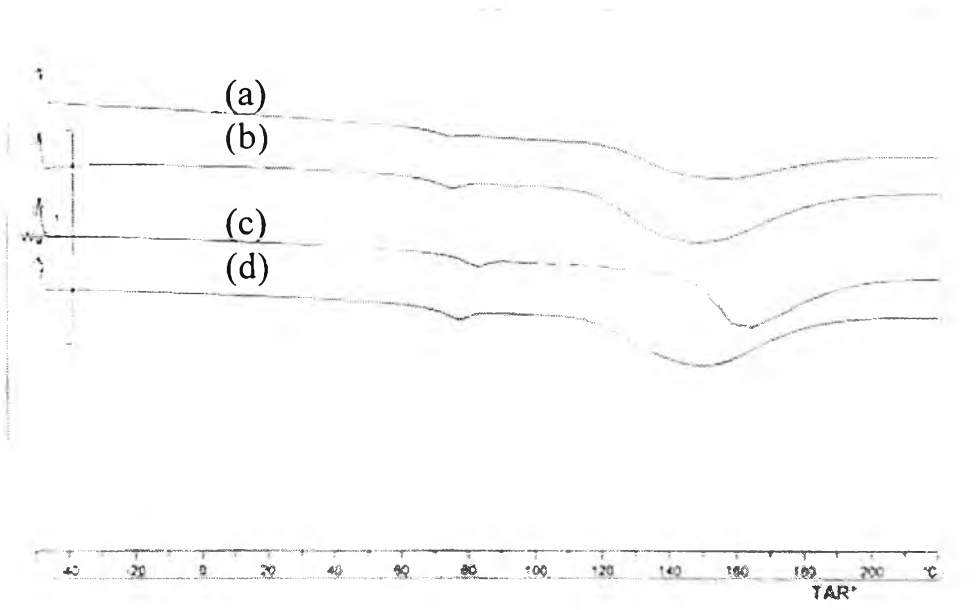


Figure 78 DSC thermograms of films in formulation E3HPC (4:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.32 mg); (b) at first month (sample weight 5.05 mg); (c) at second month (sample weight 5.02 mg); (d) at third month (sample weight 4.98 mg).

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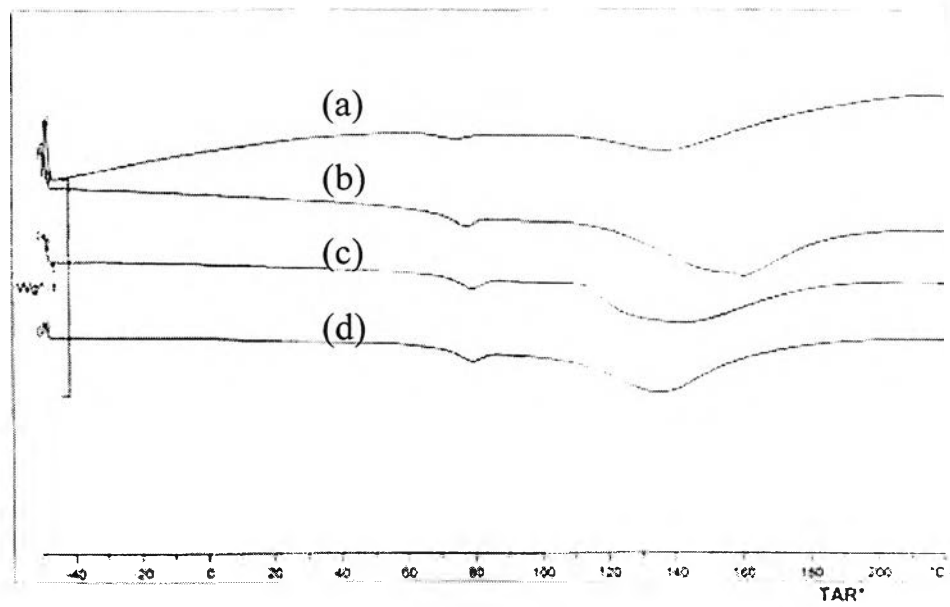

Figure 79 DSC thermograms of films in formulation E3HPC (5:1) containing *Garcinia mangostana* extract after stress condition (40 °C, 75% RH) in the temperature range of –50 to 220 °C; (a) at initial time (sample weight 5.30 mg); (b) at first month (sample weight 5.01 mg); (c) at second month (sample weight 5.05 mg); (d) at third month (sample weight 4.95 mg).

2. Chemical stability study

The amounts of mangostin containing in film formulations were assayed by HPLC at 0, 1, 2 and 3 month. The analytical method, which employed in this investigation was the HPLC method as previously described. In addition, the percentage loss of mangostin and percentage labeled amount after the exposure to heat and high humidity (40 °C, 75 %RH) at each time interval and the end of storage were also calculated.

As the results, all orally fast dissolving films appeared to be stable due to their percentage loss of mangostin was less than 10 % of the initial value (Carstensen, 1990). It was found that E3HPC (4:1) degraded with the highest extent, however, its percentage loss of mangostin was only 8.00% at the end of storage, while E3HPC (2:1) degraded with the least extent at 3.70% (Tables 40 and 41). From the analysis of data, it indicated that the remaining amount and amount of drug loss of mangostin in the all formulations after stability study were no statistically significant difference (p>0.05).

Since there was no the investigation on degradation kinetics of mangostin reported, the interpretation of the results is limited. Moreover, its degradation products have never been reported.

Table 39 Amount of mangostin in four film formulations at initial time, first, second and third month after stability study

formulation	Amount of mangostin ± SD (μg)				Percentage loss of mangostin*
	initial	1st month	2nd month	3rd month	
E3HPC (2:1)	175.91±5.95	172.41±1.75	167.58±2.59	169.41±4.24	3.70
E3HPC (3:1)	195.83±4.50	194.37±2.86	185.93±1.92	180.92±6.51	7.61
E3HPC (4:1)	186.56±3.82	184.68±0.59	177.51±2.68	171.64±3.13	8.00
E3HPC (5:1)	186.31±3.03	187.01±5.38	182.37±6.56	173.22±8.83	7.03

Table 40 Percentage labeled amount of mangostin in film in stability test

Formulation	% Labeled amount ± SD				Percentage loss of mangostin*
	initial	1st month	2nd month	3rd month	
E3HPC (2:1)	96.86±3.28	94.93±0.96	92.28±1.43	93.28±2.34	3.70
E3HPC (3:1)	107.83±2.48	107.03±1.57	102.38±1.06	99.62±3.58	7.61
E3HPC (4:1)	102.73±2.10	101.69±0.32	97.74±1.47	94.51±1.72	8.00
E3HPC (5:1)	102.59±1.67	102.97±2.96	100.42±3.61	95.38±4.86	7.03

*Percentage loss of mangostin = $\frac{\text{Initial} - \text{Final \% labeled amount} \times 100}{\text{Initial \% labeled amount}}$