CHAPTER IV

RESULTS AND DISCUSSION

1. Polymers Selection for Hydrophobic Base

1.1 Formulation of Hydrophobic Base

For preformulation studies, polyethylene (PE), polypropylene (PP) and polyethylene glycol (PEG) 6000 were employed to combine with liquid paraffin at suitable ratios in order to obtain stable mixed polymeric base with lipophillic property. PE, PP and PEG 6000 used in this study were different in physical appearance and polarity. Hydrophobic base occurred from melting process of the above polymers and mineral oil at 80 ° C. However, it was found that PP and PEG 6000 gave different results upon mixed with liquid paraffin at 80 ° C. It was noticed that when PEG 6000 and mineral oil were melted until homogeneous, after the homogeneous liquid were cooled down polyethylene glycol 6000 and mineral oil were separated from each other. These can be explained by drastic different in their polarity and chemical moiety, PEG 6000 is water soluble polymer while liquid paraffin is straight chain aliphatic hydrocarbon. In this case, adhesion force between PEG 6000 and mineral oil can not occurred and leading to the separation. In case of PP polymer, at the range of melting temperature which suit for the production the polymer was not melted. Among these three polymers, PE at molecular weight of between 1000-2000 can mix well with mineral oil in every proportion.

Hydrophobic base made from PE and mineral oil were not separated because the non polar force between PE and mineral oil was occurred. Furthermore Multimer et al., 1956 found that PE in mineral oil precipitates as small crystallites surrounded by long fibrous amorphous filaments which intermesh and produce a sponge – like structure resulting in a three dimensional lattice responsible for the gel structure.

1.2 An Evaluation of Hydrophobic Base between PE and Mineral Oil

Hydrophobic base made from PE and mineral oil were divided to 4 concentration levels; these are 1.5%, 3.0%, 4.5% and 6.0% respectively, furthermore these formulas were evaluated in following topics :

1.2.1 Viscosity at an Ambient Temperature.

After 4 levels of hydrophobic base composed of PE and mineral oil were prepared the viscosity of these hydrophobic bases were measured. The results are shown in Table 3.

Table 3 The relationship between viscosity of hydrophobic base at an ambient temperature and the percentage of PE polymer

Polyethylene polymer (%)	Viscosity (Pas.)
1.5	0.150 ± 0.01
3.0	0.217 ± 0.01
4.5	0.237 ± 0.01
6.0	0.367 ± 0.01

All values were mean ± SD of three samples

The results from Table 3 shows that the viscosity of hydrophobic bases were increased with increasing the percentage of PE polymer. In general, when the percentage of polymers were increased the viscosity of products were increased too.

1.2.2 Relationship Between the Percentage of PE Polymer and Viscosity Under Influence of Temperatures.

The effects of temperature to viscosity of hydrophobic base containing various percentages of PE are presented in Table 4 and the rheograms of viscosity which corresponding to the effect of temperature and 4 levels of hydrophobic base are illustrated in Figures 9-20.

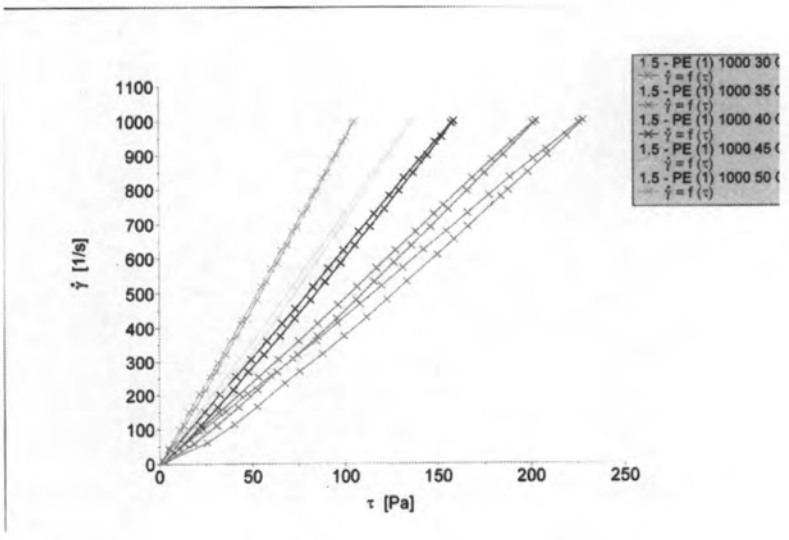


Figure 9 Rheograms of hydrophobic base containing 1.5 % PE polymer in the temperature range of 30-50 ° C

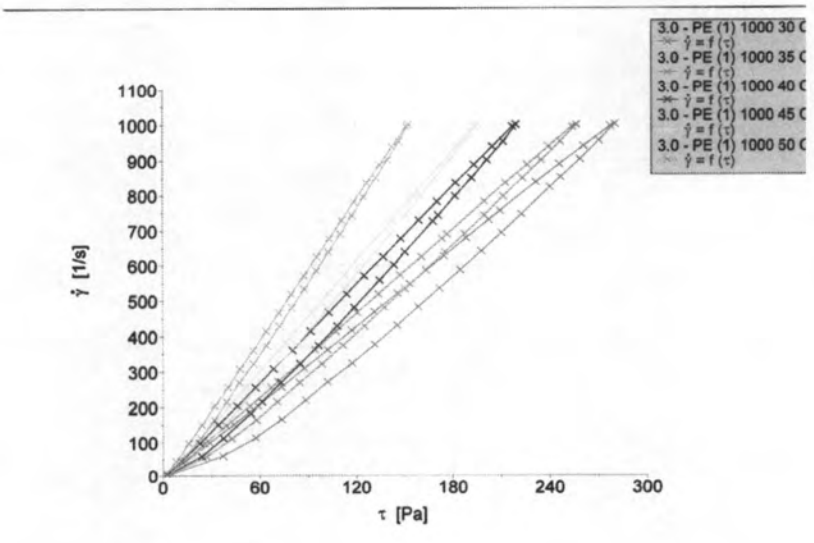


Figure 10 Rheograms of hydrophobic base containing 3.0 % PE polymer in the temperature range of 30-50 ° C

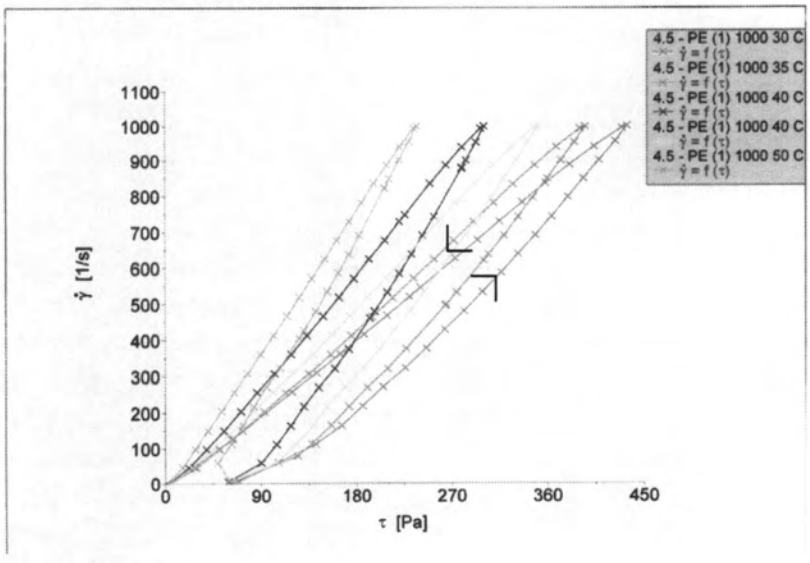


Figure 11 Rheograms of hydrophobic base containing 4.5 % PE polymer in the temperature range of 30-50 ° C

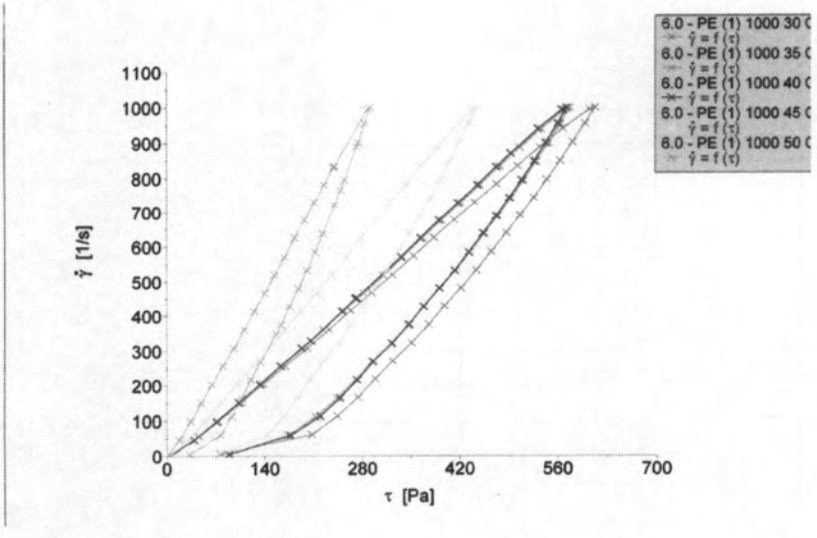


Figure 12 Rheograms of hydrophobic base containing 6.0 % PE polymer in the temperature range of 30-50 ° C

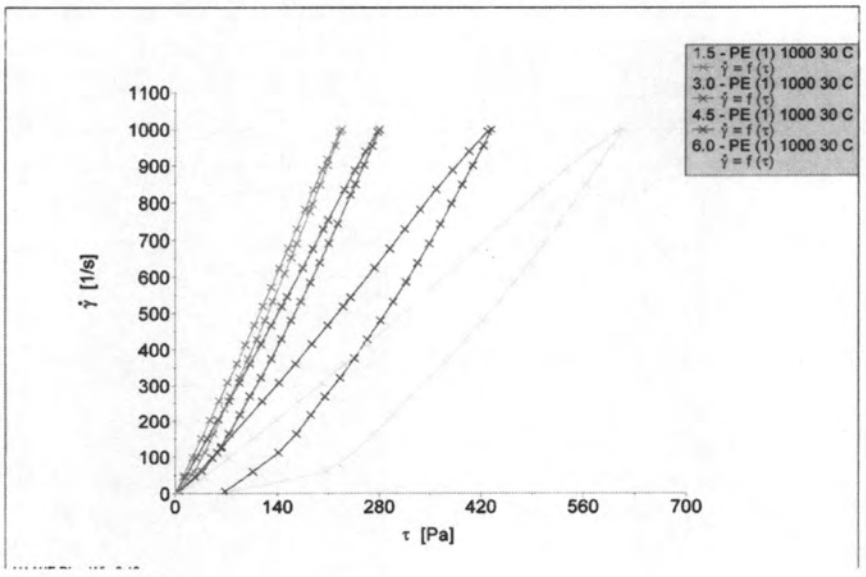


Figure 13 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at 30 ° C

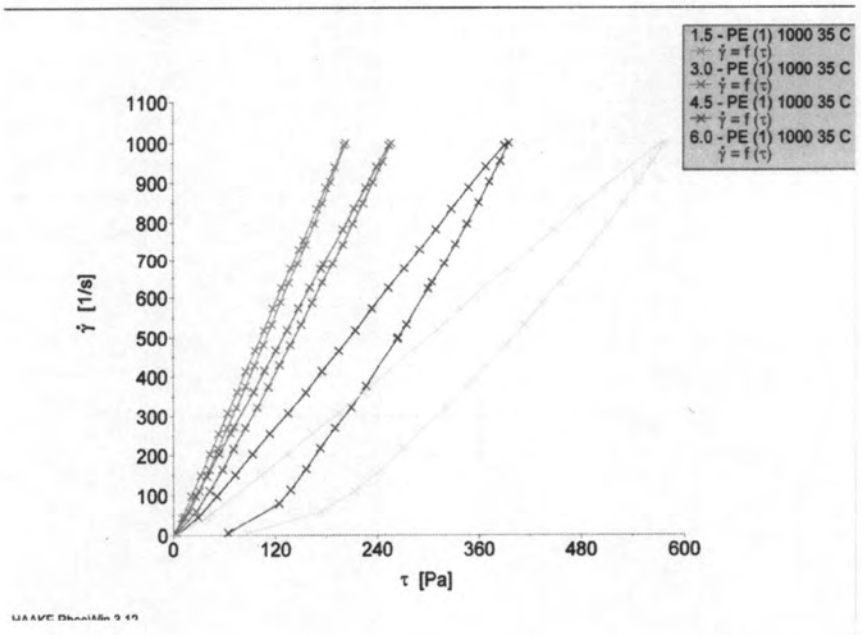


Figure 14 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at 35 ° C

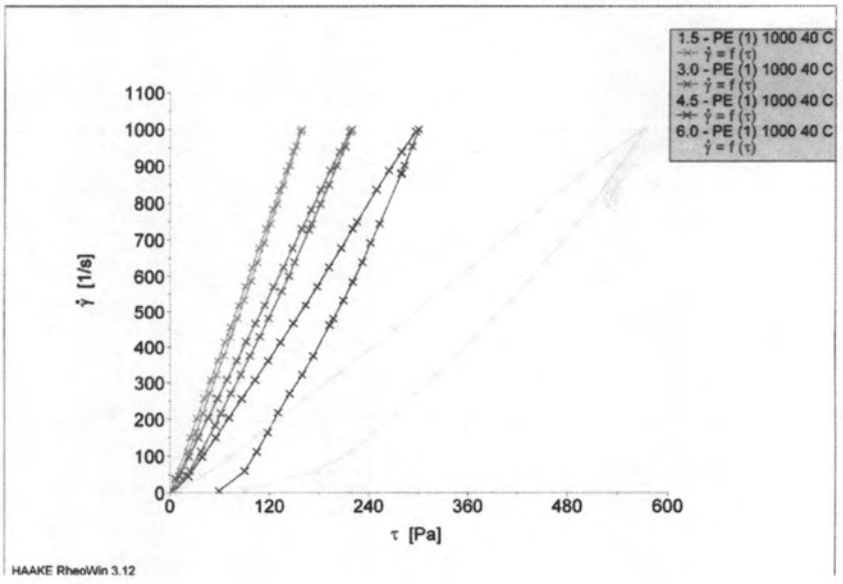


Figure 15 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at 40 ° C

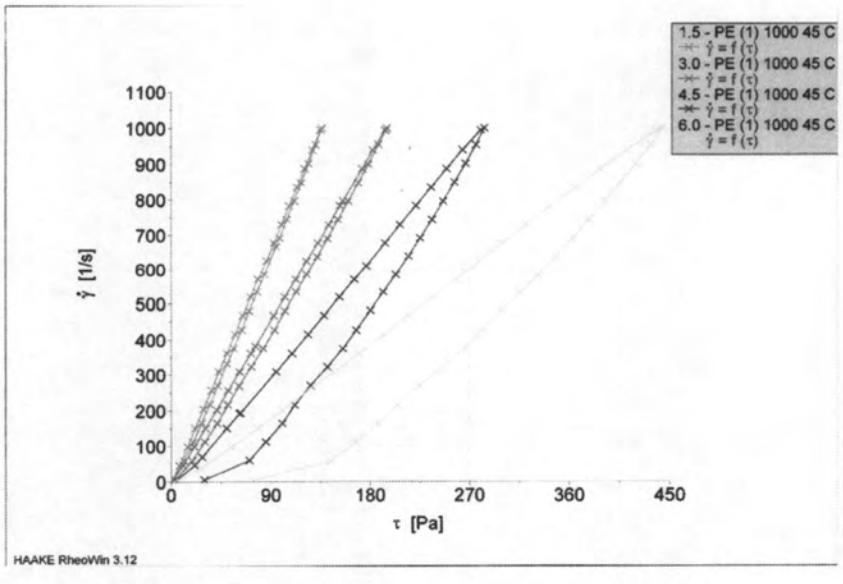


Figure 16 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at 45 ° C

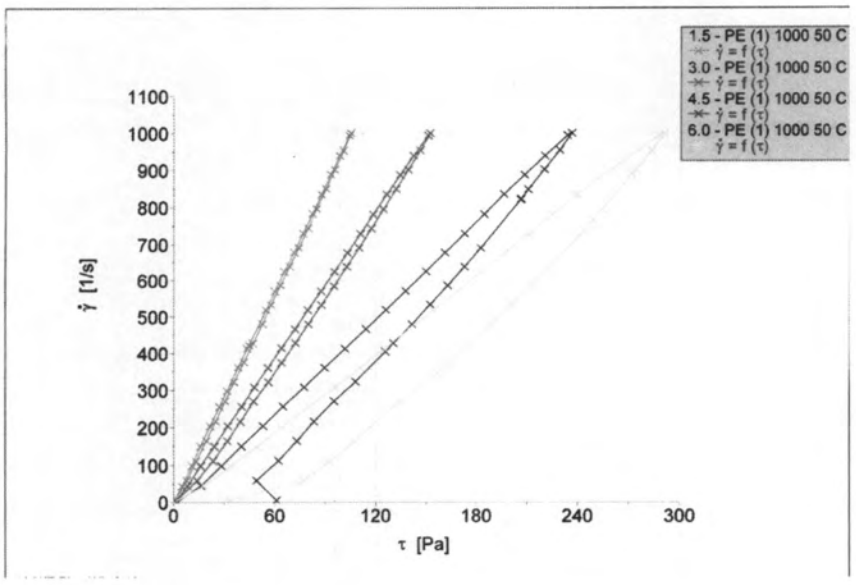


Figure 17 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at 50 ° C

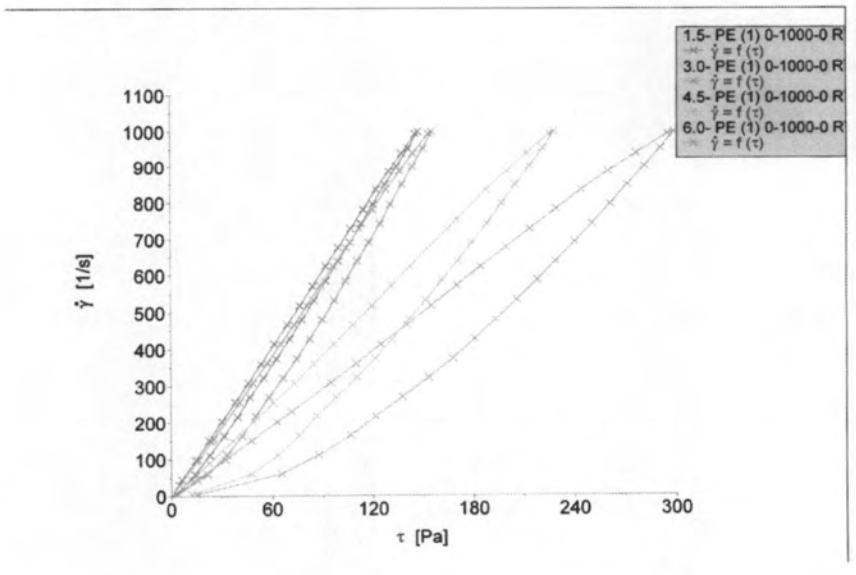


Figure 18 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer at an ambient temperature

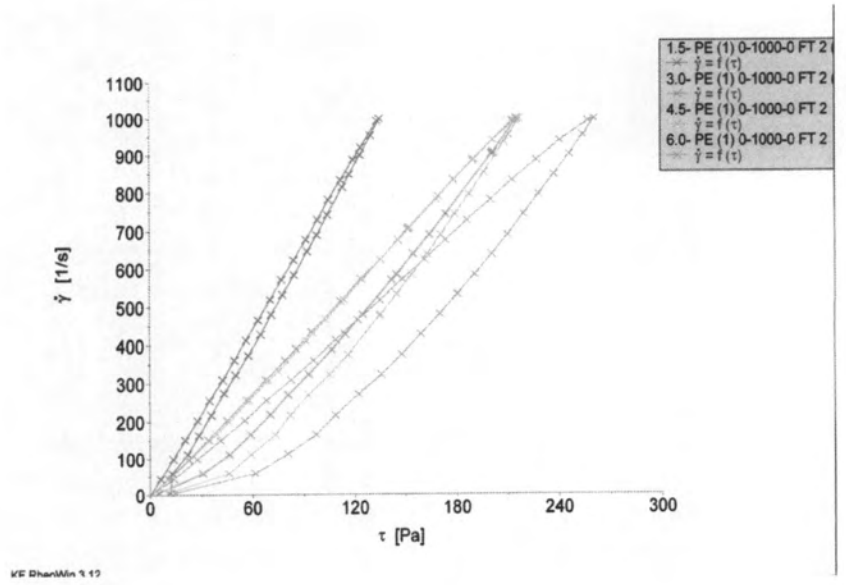


Figure 19 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer after passed freeze-thaw cycles for 5 cycles

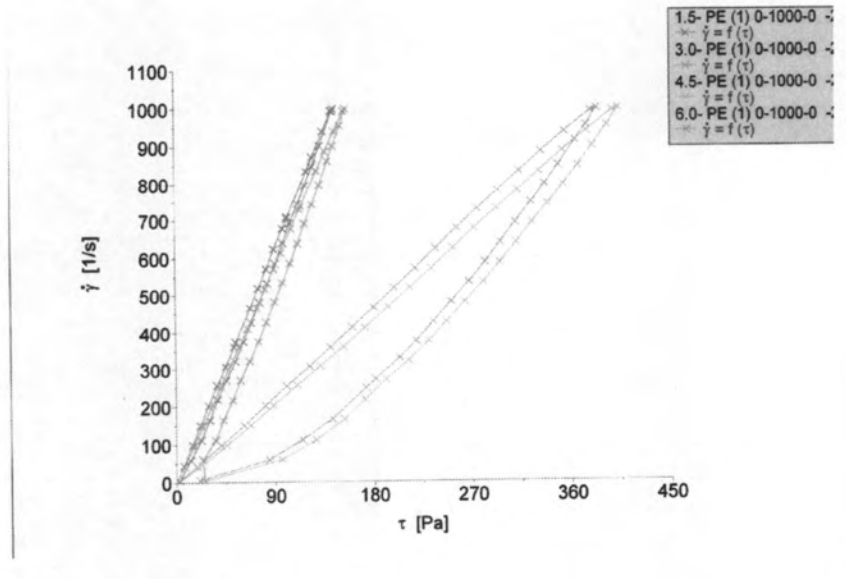


Figure 20 Comparative evaluations of the rheograms of hydrophobic base containing 1.5%, 3.0%, 4.5% and 6.0% PE polymer after passed -20 ° C

Figures 9 - 12 shows the rheograms of each level of hydrophobic base at temperatures range from 30 to 50 ° C. It is interesting to note that the loop area does not change remarkably with increasing temperature except at 6.0 % PE polymer. It considered that this was due to the resinous structure of hydrophobic base which was able to withstand upon the temperature changes over the range encountered in practice. However, these loop areas has no fundamental significance in relation to consistency behavior at a molecular level under effect of temperature (Martin et al.,1968). Figures 11 and 12 shows rheograms of hydrophobic base containing 4.5 and 6.0 % PE polymer, respectively. The rheograms of these two levels of PE polymer show pseudoplastic behavior. The upcurve of the rheogram is characterized by yield value indicating the minimum shear stress required to cause the material to flow. Below this point the material is behaving as a solid. Beyond this point the upcurve is smooth and demonstrates a pseudoplastic characteristic. The downcurve has a considerable path of its length. Rheograms of hydrophobic base containing 1.5 -6.0 % PE at temperature of 30, 35, 40, 45 and 50 ° C are presented in Figures 13-17. It seemed that rheograms of these hydrophobic bases in each temperature were not different. Loop area of hydrophobic base could be ranked as following: 6.0 % > 4.5 % > 3.0 % > 1.5 % PE polymer, respectively.

Table 4 The relationship between viscosity of hydrophobic base and the percentage of PE polymer in various temperatures

% Polyethylene	**Viscosity (Pas)**				
	Temperature				
	30 ° C	**35 ° C**	**40 ° C**	**45 ° C**	**50 ° C**
1.5	0.228	0.203	0.159	0.136	0.105
3.0	0.280	0.257	0.219	0.195	0.152
4.5	0.428	0.395	0.351	0.283	0.237
6.0	0.613	0.591	0.573	0.444	0.291

The results of this experiment showed that when the temperature of hydrophobic base was increased the viscosity of hydrophobic base was decreased, these results are in agreement with Davis et al., 1980.

1.2.3 Activation Energy of Hydrophobic Base

The viscosity data obtained from the previous studies were calculated by using the viscosity modification of Arrhenius's equation:

$$\log \eta = \log A + \frac{E}{2.303\ RT}$$

When η is viscosity of liquid, A is proportionality constant, E is energy of activation for viscous liquid, R is molar gas constant and T is absolute temperature (° C +273).

A semilog plot of viscosity as a function of percentage of PE polymer yields an approximately linear relationship as shown in Figure 21.

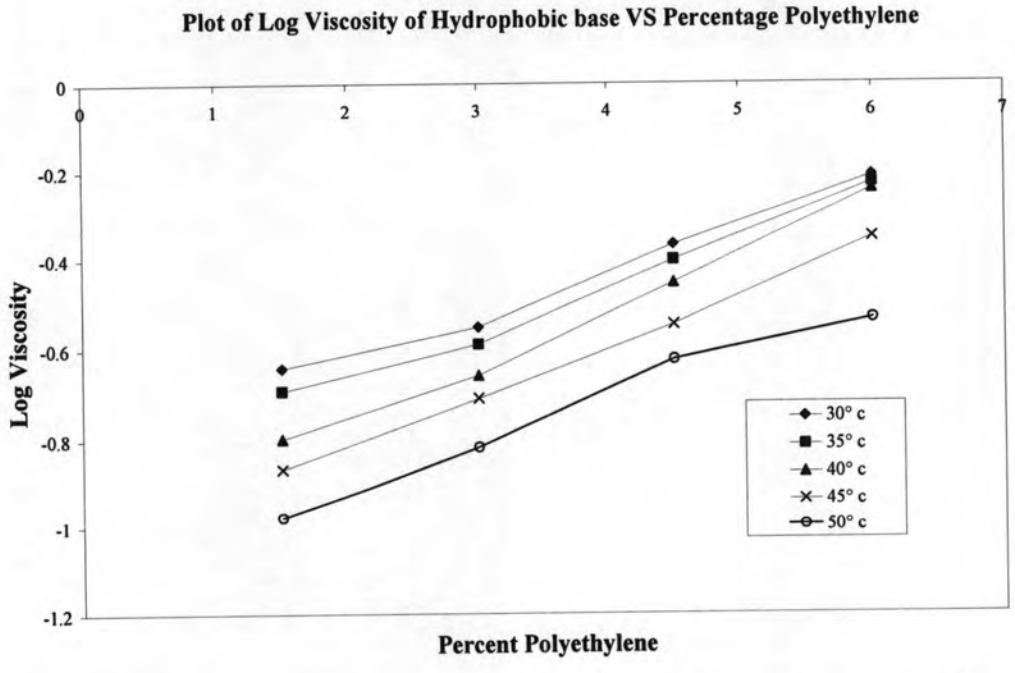


Figure 21 Plots of viscosity data obtained from continuous shear testing of hydrophobic base versus percentage of PE polymer

The results from table 5 shows relationship between the percentage of PE polymer and viscosity in term of equation that regression analysis was used to calculate the correlation of determination.

Table 5 Linear relationship between percentage of PE (PPE) and viscosity (ŋ) in term of modification of Arrhenius's equation

Temperature (° c)	Equation	Correlation of Determination (R^2)
30	Log ŋ = 0.9820 PPE - 0.8122	0.9830
35	Log ŋ = 0.1053 PPE – 0.8733	0.9860
40	Log ŋ = 0.1250 PPE – 1.0075	0.9914
45	Log ŋ = 0.1136PPE – 1.0451	0.9971
50	Log ŋ = 0.1014 PPE – 1.1198	0.9813

The viscosity data yielded linear Arrhenius type plots over the temperature range of studied were investigated in continuous shear as shown in Figure 22.

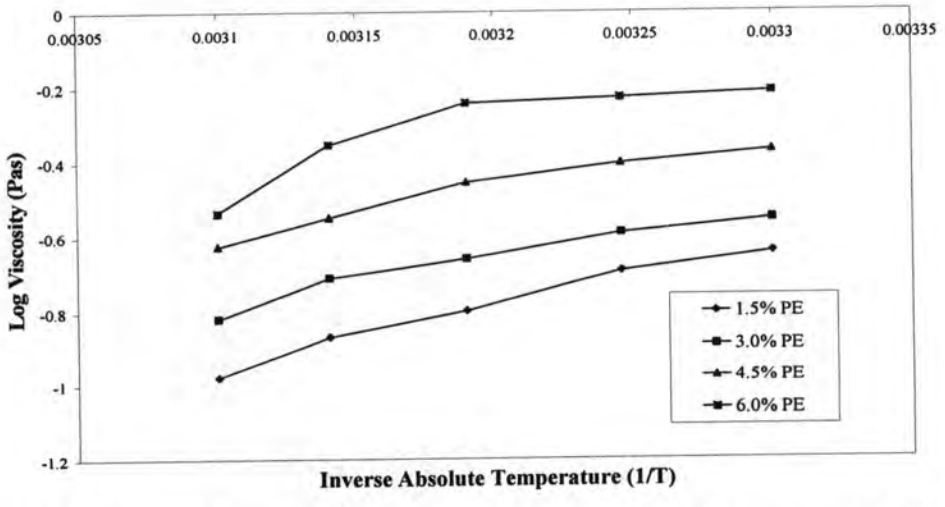


Figure 22 Arrhenius type plots of viscosity data obtained in continuous shear testing of hydrophobic base at various percentages of PE

Activation energy of these hydrophobic bases obtained from the plots are presented in Table 6.

Table 6 Activation energy values for the viscous flow of hydrophobic base as derived from continuous shear viscometry

Polyethylene (%)	ΔE value (kj / mol)
1.5	18.01
3.0	13.30
4.5	12.45
6.0	5.67

The modified Arrhenius's equation can be applied for the viscous flow of semisolid materials in order to calculate the energy barrier of the system. This based upon a lattice structure of the mixed polymer for liquid in the system containing some occupied site or holes. Without stress, molecules in liquid still unmoved because the energy barrier. Under applied stress, molecules jumped from site to site in the direction which relieves stress thus initiate viscous flow (Glasstone et al.,1941).

The activating energy values evaluated for four levels of hydrophobic base as indicated in Table 6 decreased with increasing concentration of PE polymer in hydrophobic base. This would suggest that base of low PE content behave in a similar manner to mineral oil and require a high energy of activation for complete molecular flow. In contrast, at the higher PE polymer content, hydrophobic base may exhibited segmental molecular flow which viscous flow take place by successive jumps of segment of a molecular entity instead of the whole entity so, this process required a relative lower energy of activation.

1.2.4 Physical Stability of Hydrophobic Base Obtained From Freeze – Thaw Cycle Testing

Viscosity of hydrophobic base after exposed to freeze – thaw cycle up to 5 cycles and also freeze at -20 ° C for 24 hours are shown in Tables 7 and 8.

Table 7 The relationship between viscosity of hydrophobic base and percentage of PE polymer after exposed to 5 cycles of freeze – thaw

% Polyethylene	Viscosity (Pas)
1.5	0.123 ± 0.01
3.0	0.213 ± 0.03
4.5	0.293 ± 0.03
6.0	0.363 ± 0.01

All values were mean ± SD of three samples

Table 8 The relationship between viscosity of hydrophobic base and percentage of PE polymer after freeze at -20 ° C for 24 hrs

% Polyethylene	Viscosity (Pas)
1.5	0.102 ± 0.01
3.0	0.157 ± 0.03
4.5	0.253 ± 0.08
6.0	0.233 ± 0.01

All values were mean ± SD of three samples

The results of viscosity performed in Table 7 shows that, in every levels of PE polymer content, viscosity of hydrophobic base were decreased except at 4.5 % PE polymer content. Similar to the result obtained from freeze – thaw cycle, viscosity of hydrophobic base after exposure to -20 ° C for 24 hours decreased except at 4.5 % of PE polymer content too. This result may advise that at 4.5 % PE polymer content was an appropriate percentage of PE polymer in hydrophobic base that will be employed in the next experiments.

1.2.5. Compatibility Study between Polyethylene and Mineral Oil

Differential Scanning Calorimetry (DSC) chromatogram of polyethylene, mineral oil and hydrophobic base containing 1.5 % - 6.0% PE polymer are illustrated in Figures 23-28.

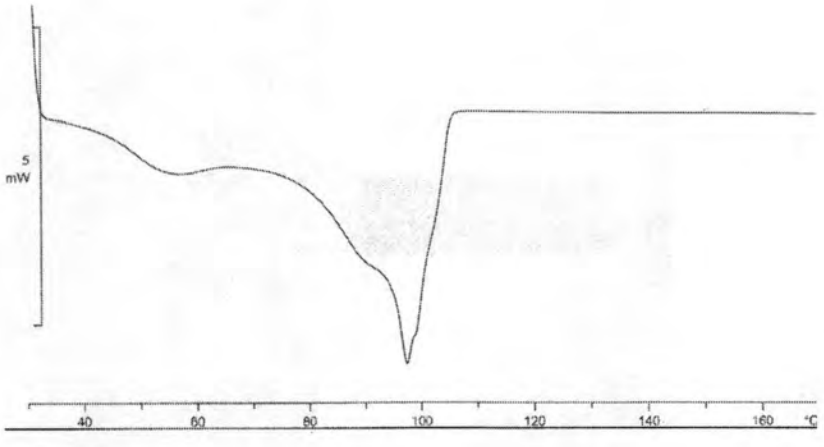


Figure 23 DSC scan of PE polymer show broad endotherm peak with onset at 97 ° C

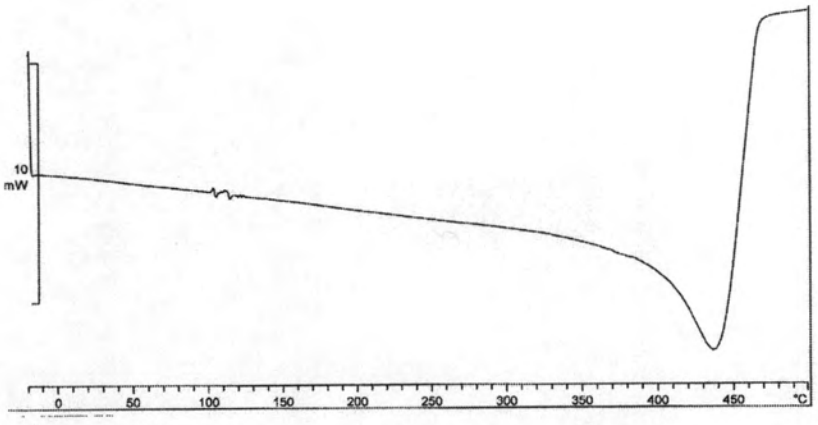


Figure 24 DSC scan of mineral oil show broad endotherm peak with onset at 440 ° C

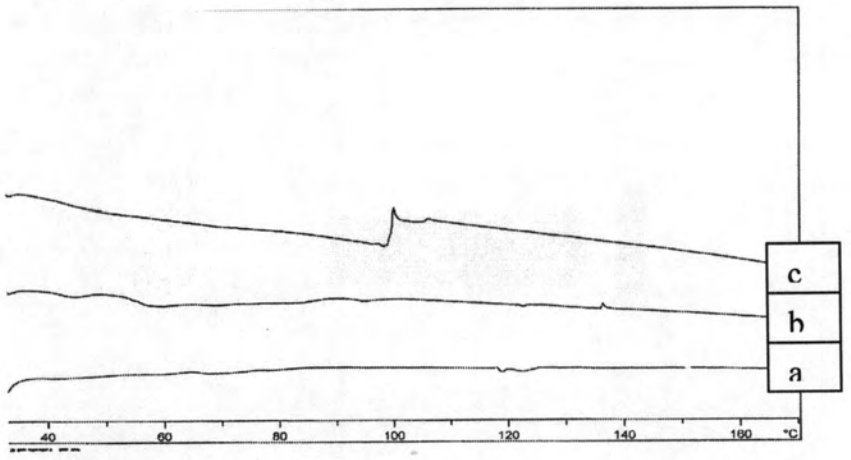


Figure 25 DSC scan of hydrophobic base containing 1.5 % PE polymer (a) at an ambient temperature, (b) after exposed to 5 cycles of freeze – thaw, (c) after freeze at -20 ° C for 24 hrs

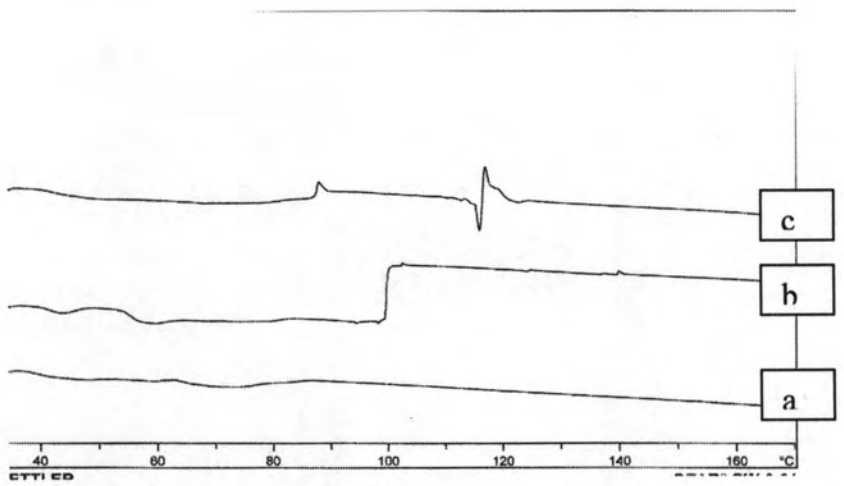


Figure 26 DSC scan of hydrophobic base containing 3.0 % PE polymer (a) at an ambient temperature, (b) after exposed to 5 cycles of freeze – thaw, (c) after freeze at -20 ° C for 24 hrs

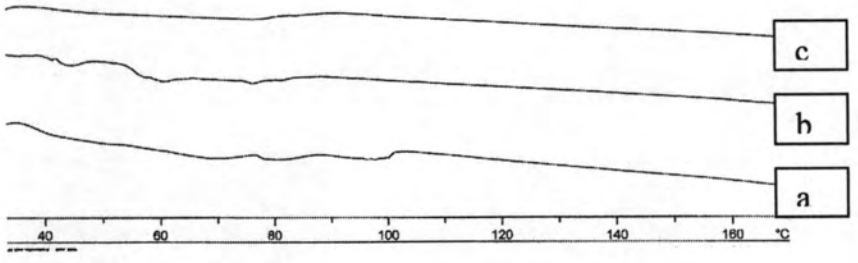


Figure 27 DSC scan of hydrophobic base containing 4.5 % PE polymer (a) at an ambient temperature, (b) after exposed to 5 cycles of freeze – thaw, (c) after freeze at -20 ° C for 24 hrs

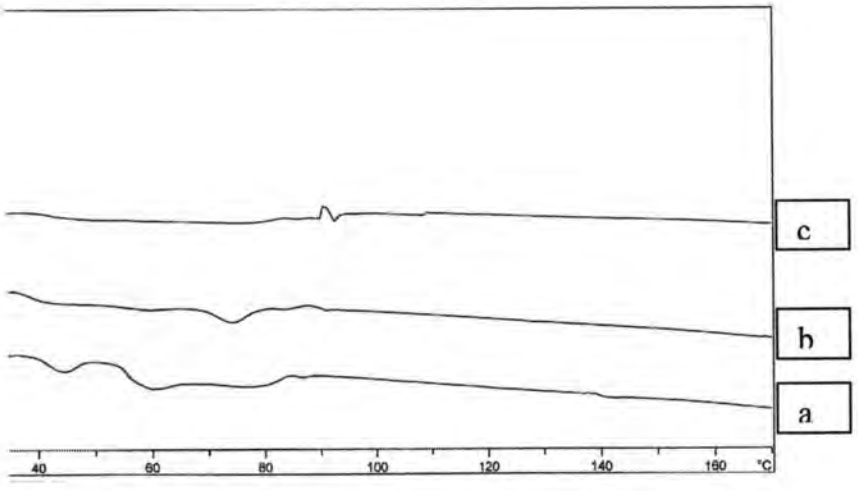


Figure 28 DSC scan of hydrophobic base containing 6.0 % PE polymer (a) at an ambient temperature, (b) after exposed to 5 cycles of freeze – thaw, (c) after freeze at -20 ° C for 24 hrs

DSC scan of PE polymer obtained from Figure 23 shows that the onset of endotherm which indicate the melting temperature of polyethylene polymer about 97 ° C. When PE polymer and mineral oil were mixed together and formed hydrophobic base DSC scan of hydrophobic base were different from PE polymer and mineral oil alone. Figure 25 shows DSC scan of 1.5 % PE polymer in hydrophobic base in every condition: (a) at an ambient temperature, (b) after exposed to 5 cycles of freeze – thaw and (c) after freeze at -20 ° C for 24 hours. The results from Figure 25 shows that the large endothermic peak of PE polymer was absent. It may be concluded that the inclusion of PE polymer and mineral oil was occurred. This phenomenon can be explained by their chemical nature of PE polymer and mineral oil. These two compounds are long chain hydrocarbon with different molecular weight. Molecular weight of PE polymer used in this study was between 1000-2000, however for mineral oil the MW is much more higher than PE polymer and containing the same constituent. In this case inclusion of PE polymer in mineral oil will occurred actually by non polar forces and probably can not detect by DSC scan. The same result of lacking PE polymer endotherm in DSC scan also appeared with 1.5 % polyethylene hydrophobic base after freeze – thaw cycles in Figure 25. Unlike 1.5 % polyethylene hydrophobic base, the DSC scan of 1.5 % polyethylene hydrophobic base treated with – 20 ° C 24 hours showed the small unidentify peak at 100 ° C as given in Figure 25.

From Figure 26 DSC scan of 3.0 % PE polymer hydrophobic base were similar to 1.5 % polyethylene hydrophobic base but different from hydrophobic base treated with freeze – thaw cycles and at -20 ° C for 24 hours.

DSC scan of 4.5 % PE polymer hydrophobic base in every condition are presented in Figure 27. It performed that thermogram of 4.5 % PE polymer treated with freeze – thaw cycles and at –20 ° C for 24 hrs were similar to 4.5 % PE polymer hydrophobic base that the peak of PE polymer were disappeared.

Figure 28 shows DSC scan of 6.0 % PE polymer in hydrophobic base at all conditions. Three DSC scans of this level of PE polymer were similar to DSC scan of hydrophobic base containing 1.5 % PE polymer but different in DSC scan that treated with -20 ° C for 24 hrs. A small unidentify exotherm peak at 90 ° C was occurred with 6.0 % PE polymer hydrophobic base treated with -20 ° C for 24 hours, however DSC scan of 1.5 % PE polymer hydrophobic base treated with -20 ° C for 24 hours also showed small unidentify exotherm peak but at 100 ° C.

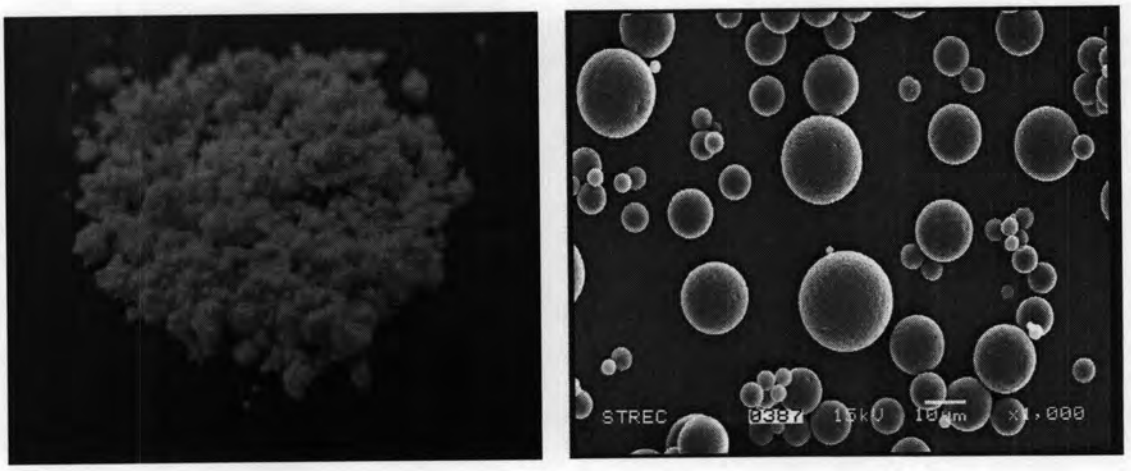
From the previous data it may be assumed that formula containing 4.5 % PE polymer was appropriate to formulate oral paste due to it physical stability as indicated by DSC scan which did not change in every condition studied.

2. Selection of Gelling Agent

2.1. Preparation and Evaluation of Chitosan Salts

Chitosan hydrochloride MW 227,000, chitosan glutamate MW 227,000, Chitosan hydrochloride MW 83,000 and chitosan glutamate MW 83,000 were prepared by spray – drying method with suitable conditions. Chitosan salt solutions were yellowish and clear. The physical appearance of chitosan salts powder related to their nature properties of materials. The physical characteristics of chitosan salts were agglomerate powder with yellowish color. The photomicrograph of chitosan salts from scanning electron microscope (SEM) are illustrated in Figures 29-32. The

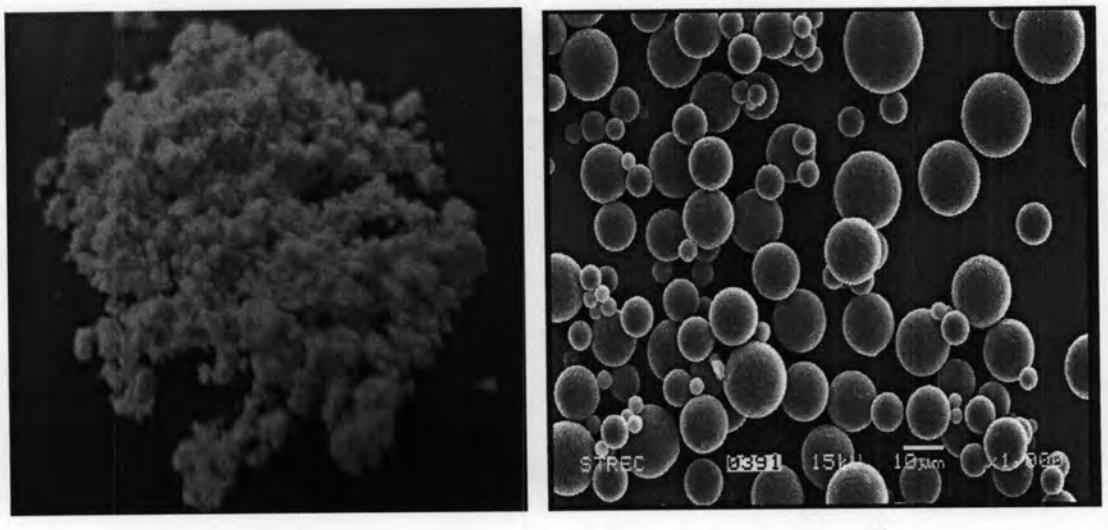
particles of chitosan salts powder from spray - dry process are round shape except for chitosan hydrochloride at MW. 227,000 which shrinking on the surface of particle was found.



A B

Figure 29 Photomicrograph of chitosan glutamate MW. 83,000

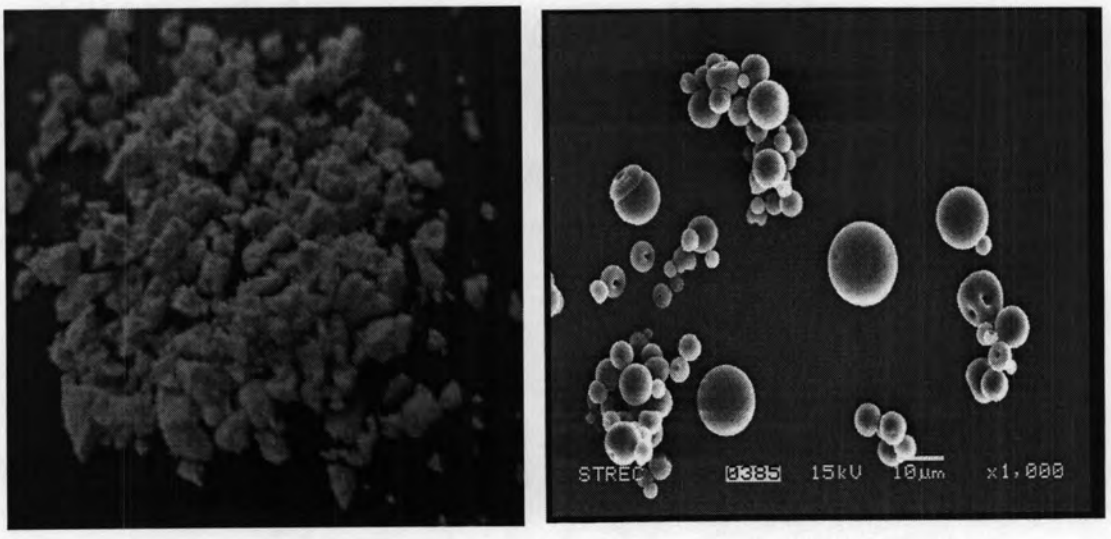
(A) Simple photograph, (B) Scanning Electron Microscope (X1000)



A B

Figure 30 Photomicrograph of chitosan glutamate MW. 227,000

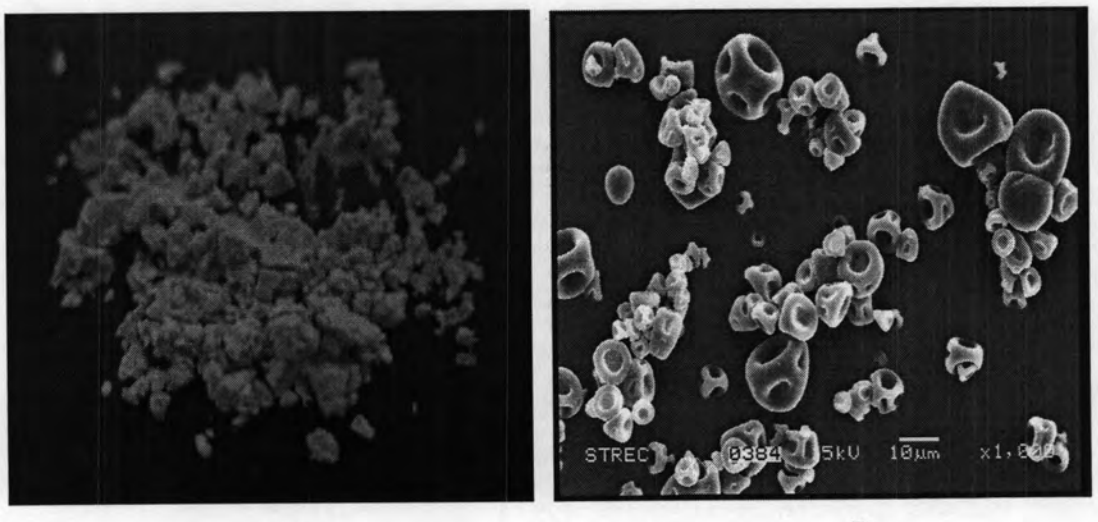
(A) Simple photograph, (B) Scanning Electron Microscope (X1000)



A B

Figure 31 Photomicrograph of chitosan hydrochloride MW. 83,000

(A) Simple photograph, (B) Scanning Electron Microscope (X1000)



A B

Figure 32 Photomicrograph of chitosan hydrochloride MW. 227,000

(A) Simple photograph, (B) Scanning Electron Microscope (X1000)

The viscosity and pH of chitosan salts solutions at 0.5 %, 1.0%, 2.0% and 3.0% concentration were measured and shown in Tables 9 and 10, respectively.

Table 9 Viscosity data of various chitosan salt solutions at 0.5%, 1.0%, 2.0% and 3.0% concentration

% Chitosan salts in solutions	Viscosity (Pas.)			
	MW. 83,000		MW. 227,000	
	Chitosan HCl	Chitosan Glu.	Chitosan HCl	Chitosan Glu.
0.5	0.0029 ± 0.0005	0.0029 ± 0.0004	0.0023 ± 0.0004	0.0112 ± 0.0003
1.0	0.0036 ± 0.0004	0.0044 ± 0.0005	0.0033 ± 0.0003	0.0144 ± 0.0007
2.0	0.0082 ± 0.0006	0.0095 ± 0.0004	0.0121 ± 0.0004	0.0547 ± 0.0003
3.0	0.0162 ± 0.0003	0.0186 ± 0.0004	0.0215 ±0.0006	0.0619 ± 0.0003

All values were mean ± SD of three samples

Table 10 The pH value of chitosan salt solutions at various concentrations of 0.5 %, 1.0%, 2.0% and 3.0%, respectively

% Chitosan salts in solutions	pH			
	MW. 83,000		MW. 227,000	
	Chitosan HCl	Chitosan Glu	Chitosan HCl	Chitosan Glu
0.5	2.58	4.13	2.79	3.98
1.0	2.27	4.08	2.48	3.87
2.0	1.89	3.84	2.03	3.83
3.0	1.69	3.76	1.77	3.71

Viscosity of all chitosan salt solutions were increased with corresponding to the concentration dissolved in solutions. In the case of viscosity, it was found that chitosan glutamate was higher than viscosity of chitosan hydrochloride in both molecular weight of 83,000 and 227,000. As was expect, viscosity of chitosan glutamate at high molecular weight was also higher than viscosity of chitosan glutamate at low molecular weight, surprisingly viscosity of chitosan hydrochloride at low molecular weight and at 0.5 and 1.0 % was found to be higher than chitosan hydrochloride at high molecular weight also at 0.5 and 1.0% solutions but were not at 2.0 and 3.0 %. In conclusion, the viscosity of chitosan glutamate MW 227,000 was the highest in this study. Relationship between viscosity of chitosan salts and its percentage are illustrated in Figure 33.

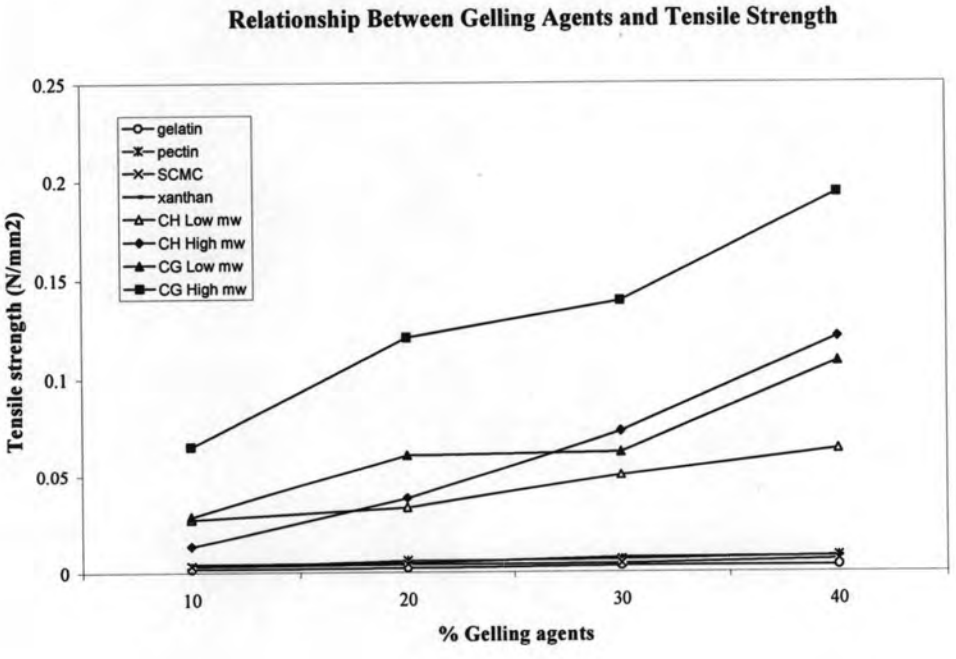


Figure 33 Plots of viscosity data obtained from continuous shear testing of chitosan salts solutions as a function of concentration

The pH values of chitosan salt solutions that presented in Table 10 show that when concentration of chitosan salts in solutions was increased the pH values of solutions were decreased. It was found that the pH value of chitosan glutamate MW. at of 83,000 and 227,000 were the same and were higher than chitosan hydrochloride. This result was attributed from pK_a of hydrochloric acid (~1) that lower than pK_a of glutamic acid (pK_a 1 = 2.19, pK_a 2 = 4.25, carboxylic; pK_a 3 = 9.67, amino). So, in this case salt of strong acid will contribute to lower pH value than salt of weak acid. In addition, the pH values of chitosan hydrochloride at high and low molecular weight were the same.

2.2 Characterization of Chitosan Salts by FT-IR

Figure 34 shows the FT-IR spectra of the sample under study.

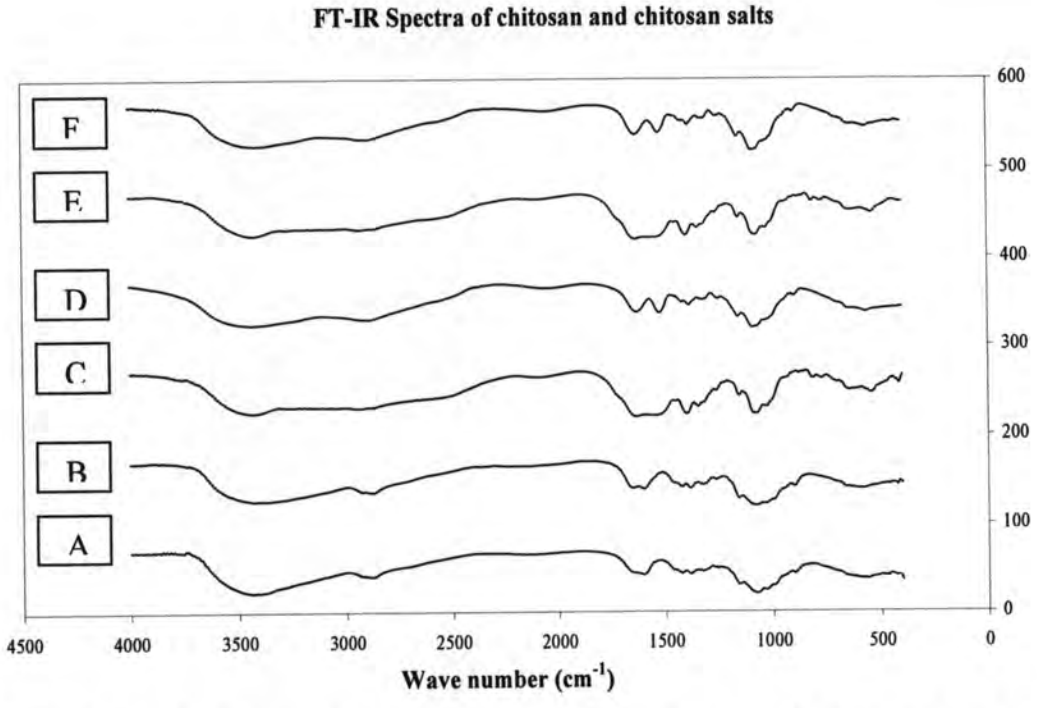


Figure 34 FT-IR Spectra of chitosan and chitosan salts : (A) chitosan MW. 83,000, (B) chitosan MW. 227,000, (C) chitosan glutamate MW. 83,000, (D) chitosan HCl MW. 83,000, (E) chitosan glutamate MW. 227,000 and (F) chitosan HCl MW. 227,000

The FT-IR spectra of chitosan depict characteristic absorption bands at 3431, 2916 and 2877/cm., which represent the presence of OH group, CH_2 and CH_3 group (aliphatic hydrocarbon group). The amino group has a characteristic absorption band in the region of 3400-3500/cm.which must have been masked by absorption band due to OH group (Shanmugasundaram et al., 2001). Chitosan showed the characteristic band of the amino group at 1651/cm. In the spectrum of spray-dried chitosan hydrochloride both molecular weight of 83,000 and 227,000 the characteristic absorption band of chitosan at about 1651/cm. ($-NH_2$) disappears giving rise to two new bands located at 1628 and 1518/cm. This behavior reflected an ionic interaction between the amino group of chitosan base and hydronium ion of hydrochloric acid by protonation of the amino groups. In addition, the spectrum of spray-dried chitosan glutamate showed a large peak ($-NH_2$) at 1629/cm. The large shift of these vibrations to higher wavenumbers compared with the usual wavenumbers of the amino group proves the formation of a carboxylate between the $-COO^-$ group of acids and $-NH_3^+$ group of chitosan (Lorenzo-Lamosa et al., 1998). Consequently, it seemed reasonable to conclude that chitosan was ionically crosslinked with the acids (Orienti et al., 2002).

2.3 An Evaluation of Physical Characteristics and Compatibility of Gelling Agents in Hydrophobic Base

Eight types of gelling agents were utilized in this study: Sodium Carboxy Methyl Cellulose (SCMC), Gelatin, Pectin, Xanthan Gum, Chitosan HCL MW 83,000, Chitosan Glutamate MW. 83,000, Chitosan HCL MW 227,000 and Chitosan Glutamate MW.227,000.

2.3.1 Moisture Adsorption Study

2.3.1.1. Saturated salt solutions of potassium nitrate (KNO_3)

From the data obtained in Figure 35 shows the degree of moisture adsorption of mixture of hydrophobic base and gelling agents at various concentrations between 0.51-2.12 %. These results could be ranked as follow : Sodium Carboxy Methyl Cellulose (SCMC) > Xanthan gum > Chitosan glutamate MW.83,000

> Chitosan glutamate MW.227,000 > Chitosan hydrochloride MW.83,000 > Chitosan hydrochloride MW.227,000 > Pectin > Gelatin. From these result, SCMC could absorb moisture from environment better than other gelling agents.

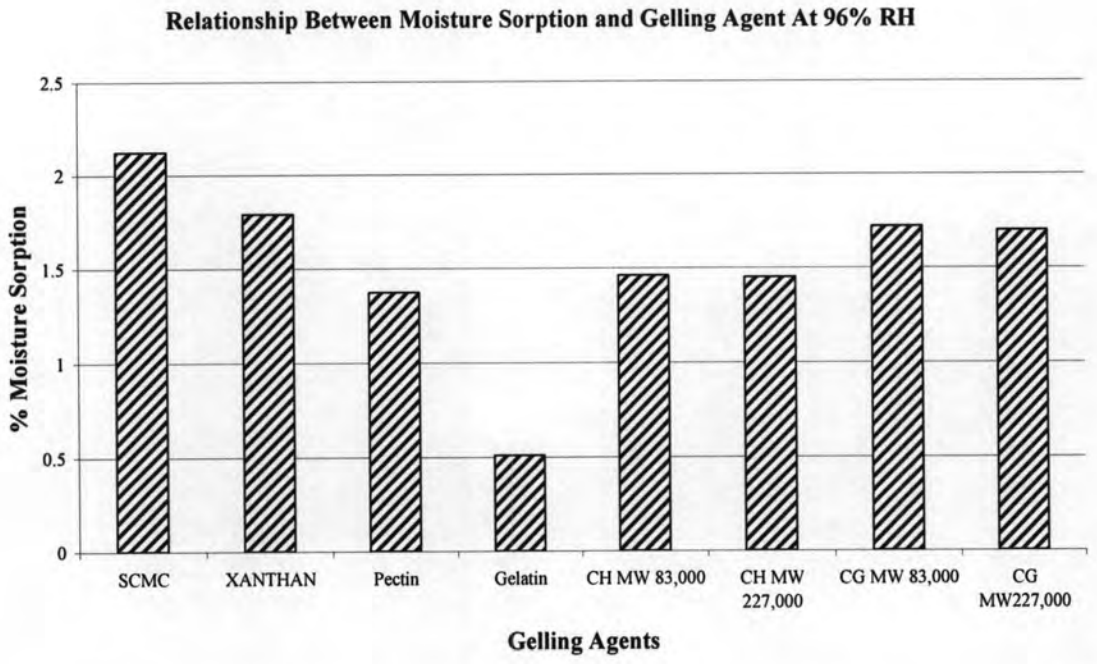


Figure 35 The percentage of moisture adsorption of various gelling agents at 96% relative humidity

2.3.1.2. Saturated salt solutions of sodium chloride (NaCl)

The percent of moisture adsorption of the mixture of hydrophobic base and gelling agents at 75% RH are given in Figure 36 could be ranked in the ordered as Sodium Carboxy Methyl Cellulose (SCMC) > Chitosan glutamate MW.83,000 >Xanthan gum ~ Chitosan hydrochloride MW.227,000 > Chitosan glutamate MW.227,000 > Chitosan hydrochloride MW.83,000 > Pectin > Gelatin. These data showed that SCMS was greater than other gelling agents in hygroscopicity.

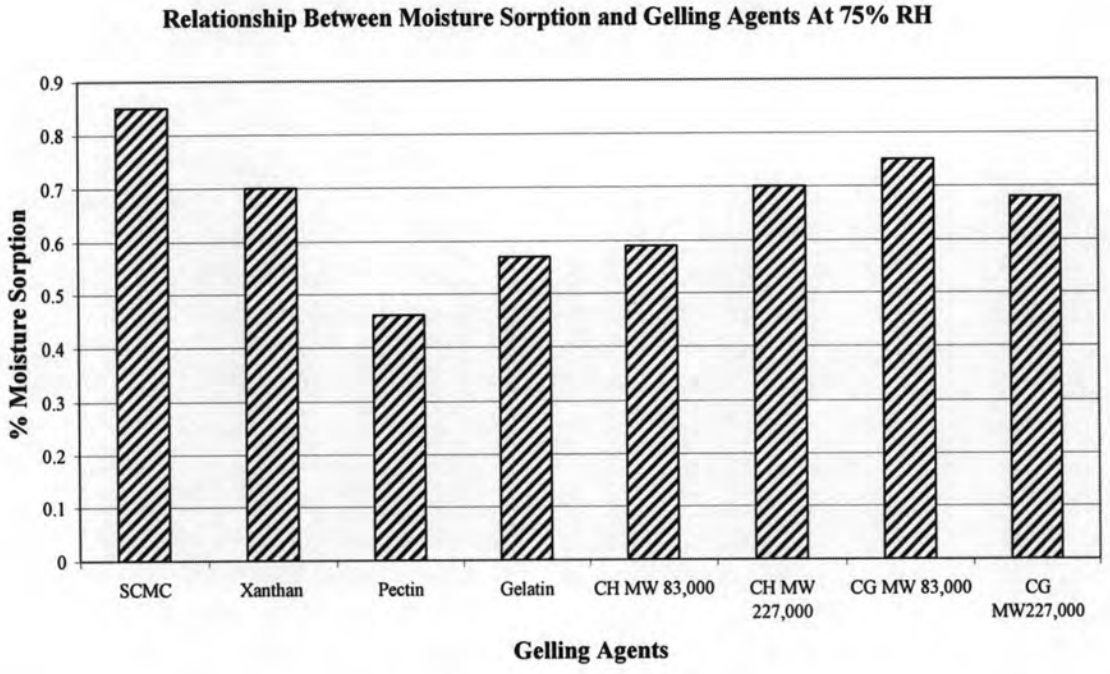


Figure 36 The percentage of moisture adsorption of various gelling agents at 75% relative humidity

2.3.1.3. Saturated salt solutions of magnesium carbonate ($Mg(NO_3)_2$)

From Figure 37 shows the percentage of moisture adsorption of the mixture of hydrophobic base and gelling agents could be ranked as follows: Chitosan hydrochloride MW.227,000 > Xanthan gum > Chitosan glutamate MW.227,000 > Sodium Carboxy Methyl Cellulose (SCMC) > Chitosan glutamate MW.83,000 > Chitosan hydrochloride MW.83,000 > Pectin ~ Gelatin. From these data the percentage of moisture adsorption of various gelling agents were between 0.05 - 0.19 %.

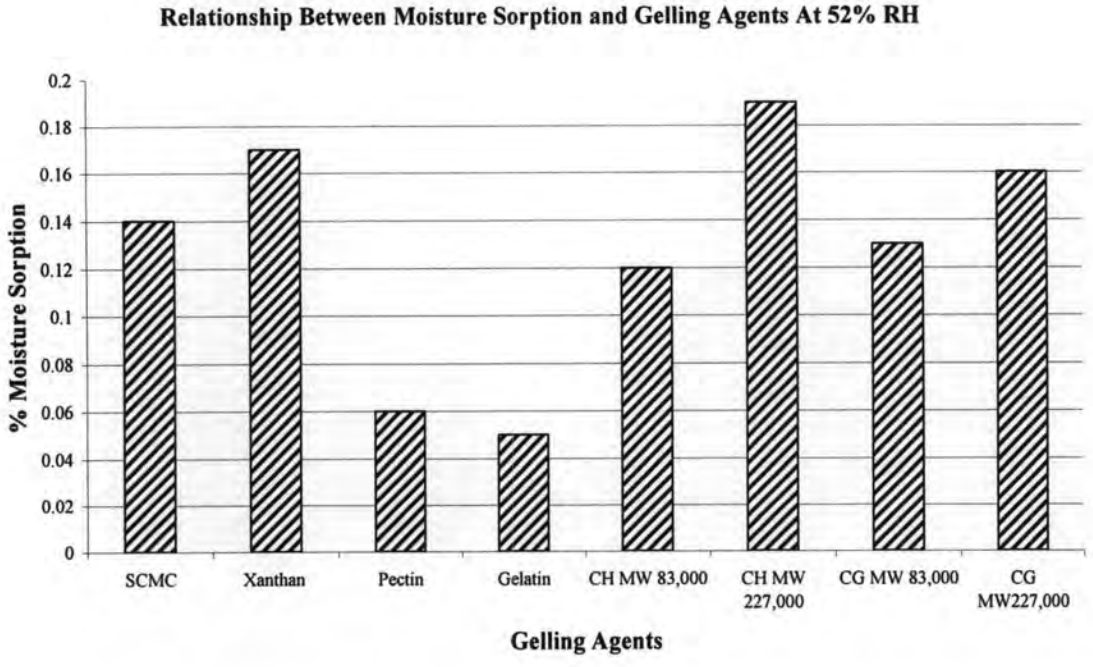


Figure 37 The percentage of moisture adsorption of various gelling agents at 52% relative humidity

2.3.1.4. Saturated salt solutions of Potassium Acetate (CH_3COOK)

In this experiment, the percentage of the moisture adsorption of mixture of hydrophobic base and gelling agents were not increased. These results indicated that relative humidity at condition of this study is lower than critical relative humidity of hydrophobic base mixed with gelling agents. In this case no gain or loss of moisture of the mixed base have been observed.

From data obtained from topic of 2.3.1.1 – 2.3.1.4 at 96 % RH and 75 % RH SCMC was gelling agent that could adsorb moisture better than other gelling agents. At 52 % RH Chitosan hydrochloride MW.227,000 could adsorb moisture greater than the other gelling agents but in this condition degree of moisture adsorption was not much different (0.05-0.19). From this experiment it could be conclude that SCMC seem to be the best gelling agent for moisture adsorption property when compared with the others.

Structure of polymer associate with hydrophilic or hydrophobic functional moieties for moisture adsorption. In general, polymer that have hydrophilic group such as salt form, amino group, carboxyl group and hydroxyl group will show higher adsorption property. On the other hand, the more alkyl group and the less polar group will show the lower water adsorption property. SCMC showed the great moisture sorption than other gelling agents due to it have a lot of polar group (i.e sodium salt, OH groups) in molecular structure than other gelling agents. The polar groups in molecule led to increase in the percentage moisture adsorption.

Data obtained from moisture adsorption of gelling agents in saturated salts solution at 52, 75 and 96 % RH can be illustrated in relationship between weigh gained of mixture of hydrophobic base and each gelling agent as a function of time that shown in Figures 38-45.

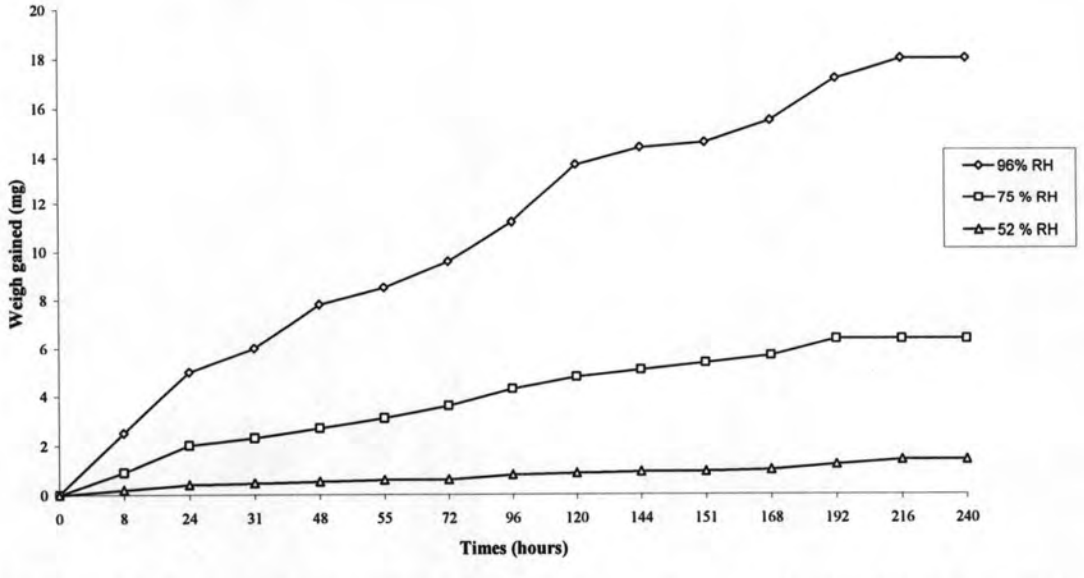


Figure 38 Relationship between weigh gained of mixture of hydrophobic base and SCMC as a function of time after storage at various relative humidities

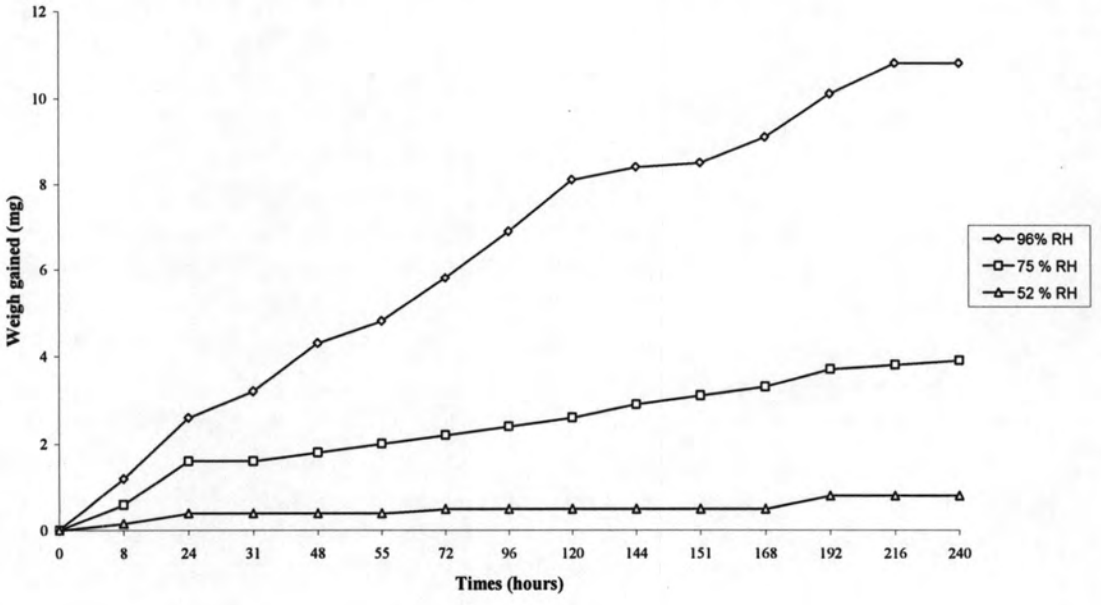


Figure 39 Relationship between weigh gained of mixture of hydrophobic base and pectin as a function of time after storage at various relative humidities

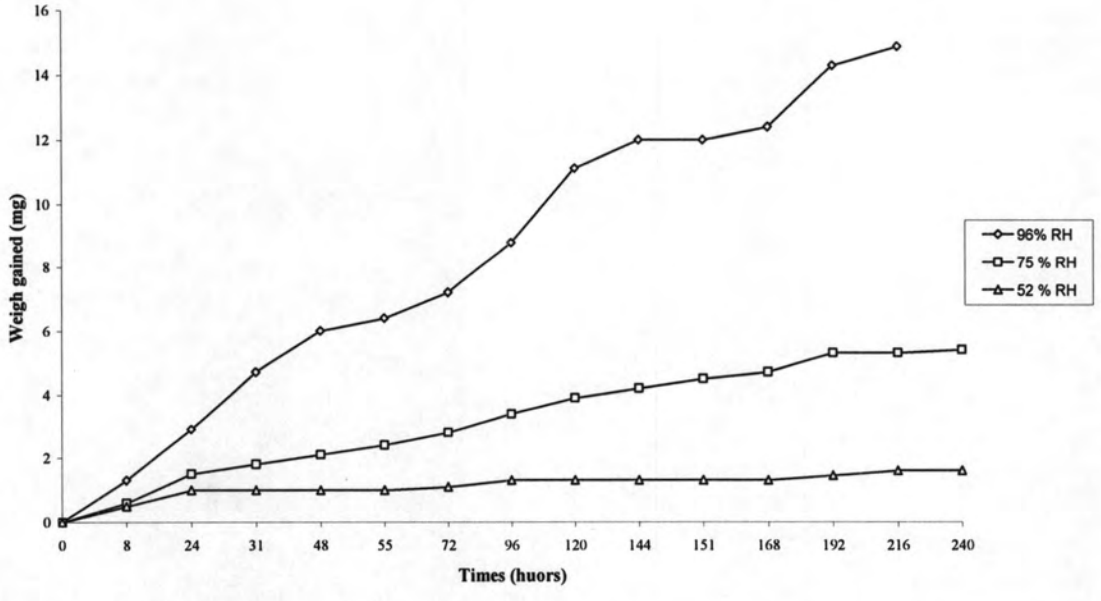


Figure 40 Relationship between weigh gained of mixture of hydrophobic base and xanthan gum as a function of time after storage at various relative humidities

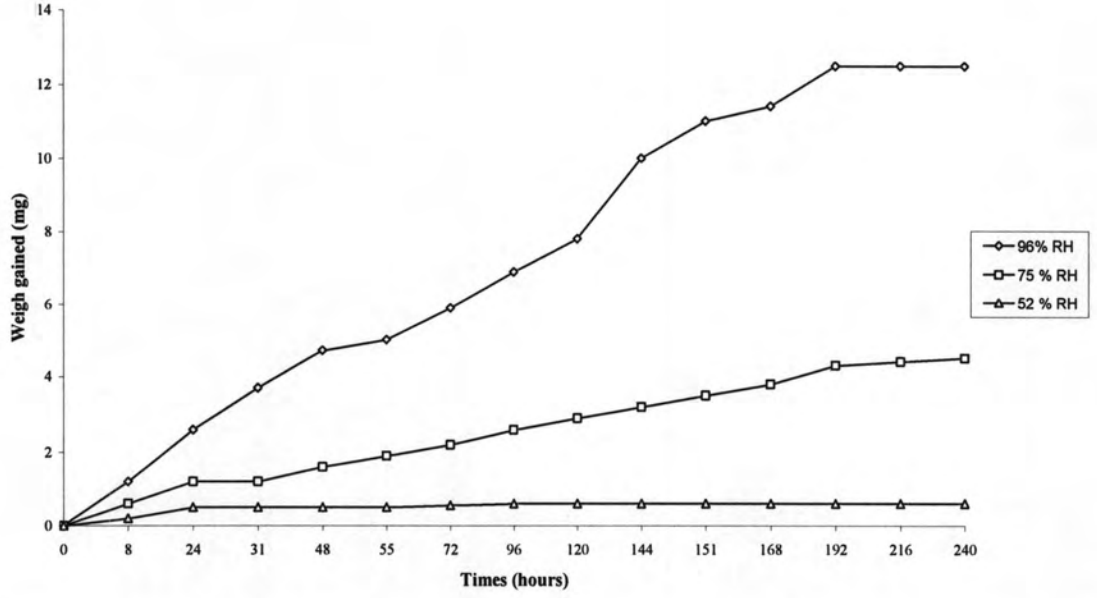


Figure 41 Relationship between weigh gained of mixture of hydrophobic base and gelatin as a function of time after storage at various relative humidities

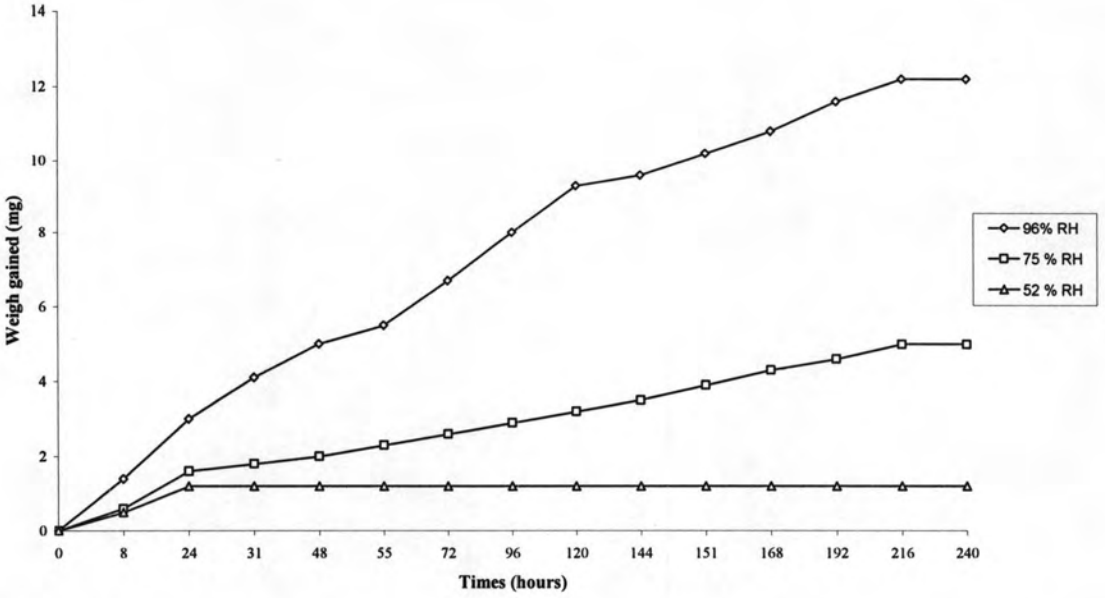


Figure 42 Relationship between weigh gained of mixture of hydrophobic base and chitosan hydrochloride MW 83000 as a function of time after storage at various relative humidities

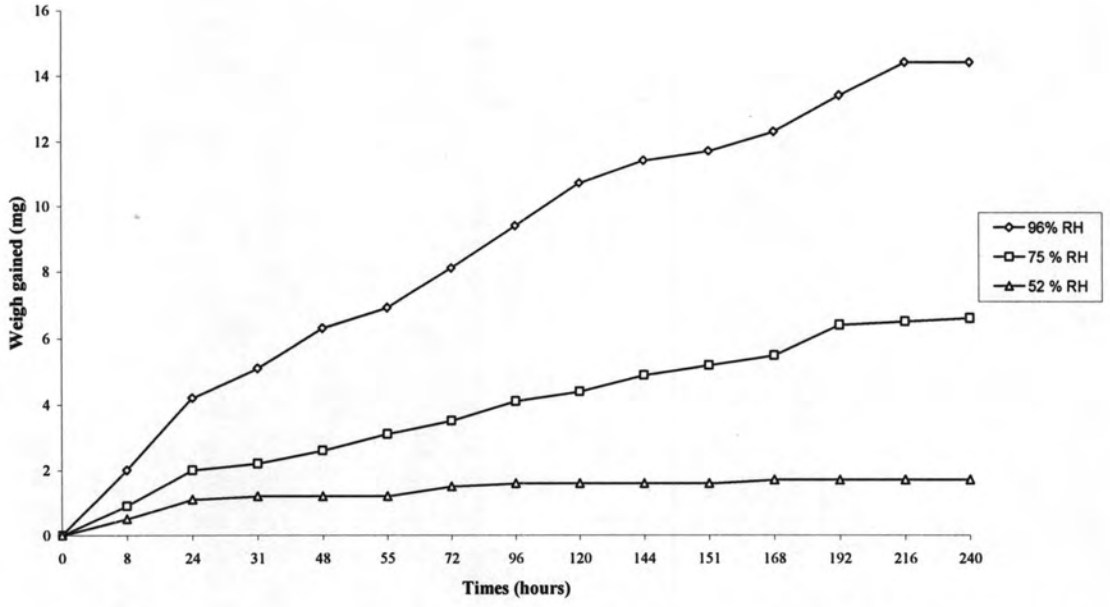


Figure 43 Relationship between weigh gained of mixture of hydrophobic base and chitosan hydrochloride MW 227000 as a function of time after storage at various relative humidities

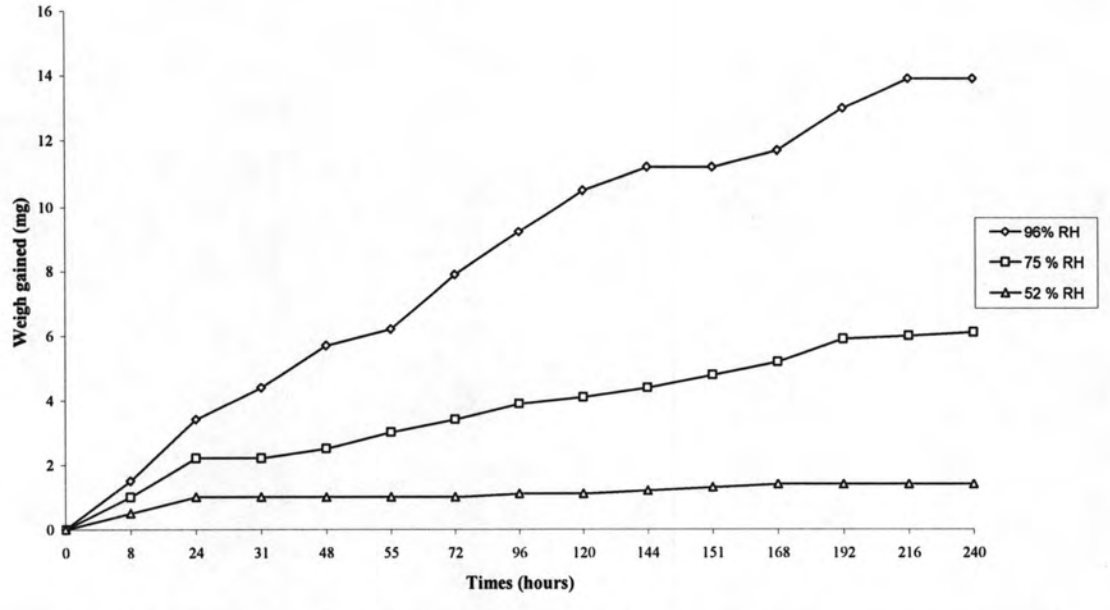


Figure 44 Relationship between weigh gained of mixture of hydrophobic base and chitosan glutamate MW 83000 as a function of time after storage at various relative humidities

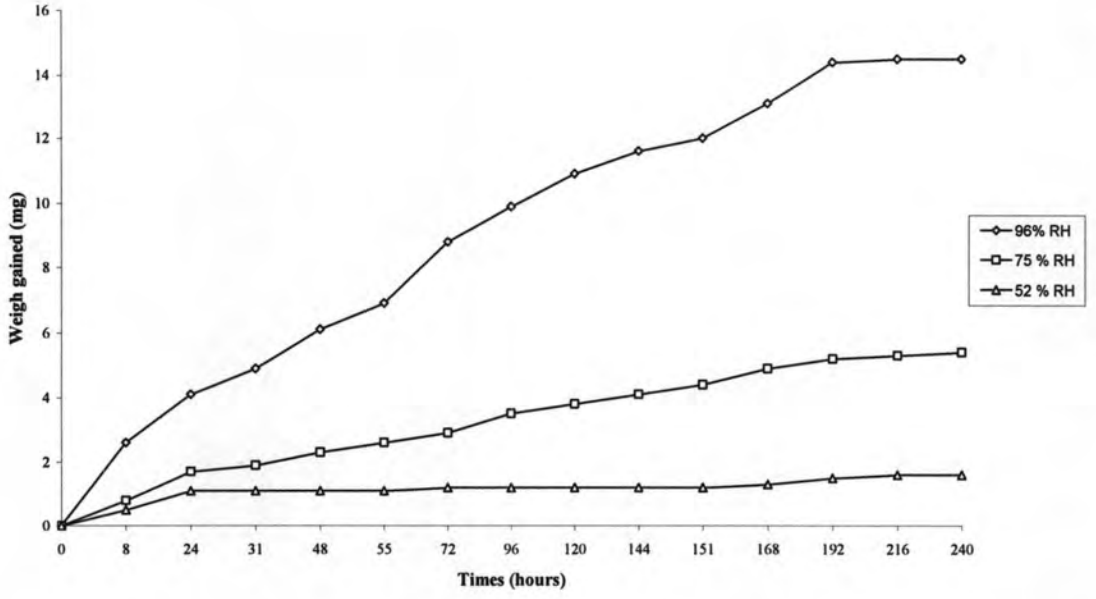


Figure 45 Relationship between weigh gained of mixture of hydrophobic base and chitosan glutamate MW 227000 as a function of time after storage at various relative humidities

The results from Figures 38-45 shows that the weigh of mixture of 80% hydrophobic base and 20% of each gelling agent were increased in every percentage relative humidity studied. At 96 % RH the weigh of mixture of hydrophobic base and each gelling agent were highest increased and the weigh of mixture of hydrophobic base and each gelling agent at 75 %RH were higher than that of mixture of hydrophobic base and each gelling agent at 52 %RH.

2.3.2 Tensile Strength Measurement of Hydrophobic Base Containing Gelling Agents

The tensile strength of mixture of hydrophobic base and gelling agents are showed in Table 11 and relationship between tensile strength and gelling agents are also illustrated in Figure 46.

Table 11 Tensile strength of hydrophobic base containing gelling agents

Ingredient (%w/w)	G 1	G 2	G 3	G 4	P 1	P 2	P 3	P 4	S 1	S 2	S 3	S 4
Gelatin	10	20	30	40	-	-	-	-	-	-	-	
Pectin	-	-	-	-	10	20	30	40		-	-	
SCMC	-	-	-	-	-	-	-	-	10	20	30	40
Hydrophobic base	90	80	70	60	90	80	70	60	90	80	70	60
Tensile strength	0.0016	0.0022	0.0032	0.0034	0.003	0.006	0.0065	0.0084	0.0032	0.0041	0.0044	0.0064

Ingredient (%w/w)	X 1	X 2	X 3	X 4	CGL 1	CGL 2	CGL 3	CGL 4	CHL 1	CHL 2	CHL 3	CHL 4
Xanthan gum	10	20	30	40	-	-	-	-	-	-	-	-
Chitosan glu. mw. 83,000	-	-	-	-	10	20	30	40	-	-	-	-
Chitosan HCl mw. 83,000	-	-	-	-	-	-	-	-	10	20	30	40
Hydrophobic base	90	80	70	60	90	80	70	60	90	80	70	60
Tensile strength	0.0042	0.0049	0.0076	0.008	0.0289	0.061	0.0625	0.1083	0.0274	0.0334	0.0503	0.064

Table 11 Tensile strength of hydrophobic base containing gelling agents (continue)

Ingredient (%w/w)	CGH 1	CGH 2	CGH 3	CGH 4	CHH 1	CHH 2	CHH 3	CHH 4
Chitosan glu. mw. 227,000	10	20	30	40	-	-	-	-
Chitosan HCl mw. 227,000	-	-	-	-	10	20	30	40
Hydrophobic base	90	80	70	60	90	80	70	60
Tensile strength	0.0656	0.1205	0.1389	0.1938	0.0137	0.0381	0.0732	0.1205

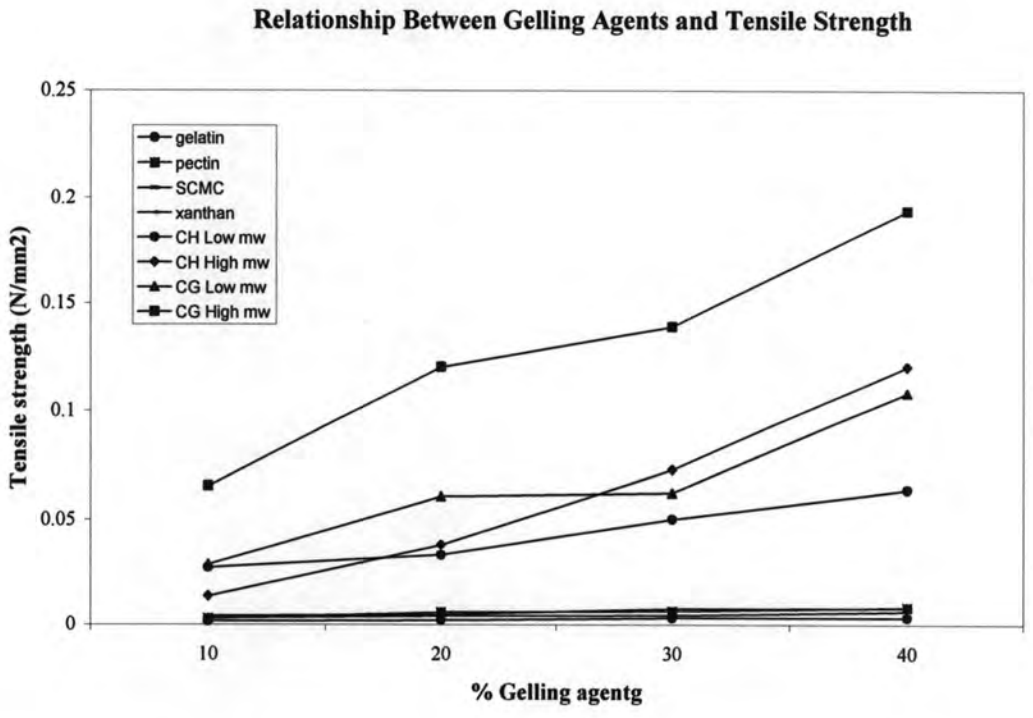


Figure 46 Relationship between tensile strength and concentration of gelling agents in the hydrophobic base

The tensile strength of gelling agents were between 0.0016-0.1938 N/mm^2. These could be ranked as follows: Chitosan glutamate MW.227,000 > Chitosan hydrochloride MW.227,000 > Chitosan glutamate MW.83,000 > Chitosan hydrochloride MW.83,000 >> Pectin > Xanthan gum > Sodium Carboxy Methyl Cellulose (SCMC) > Gelatin.

The tensile strength of the same type of gelling agent was increased with increasing the percentage of gelling agent in mixture. The mixture containing 40% of gelling agents showed the highest tensile strength in every group of gelling agents. It was found that tensile strength of chitosan salts (glutamate MW.227,000, hydrochloride MW.227,000, glutamate MW.83,000 and hydrochloride MW.83,000) were higher than tensile strength of the other gelling agents used in this study. In addition, chitosan glutamate MW.227,000 showed the highest tensile strength when compared with the others.

2.3.3 Swelling Index Test of Various Gelling Agents

In this experiment when purified water was added to cylinder containing gelling agents, gelling agents will adsorp water and swell to give a higher volume and were observed during the first 10 minute of the test. After nearly the maximum adsorption capacity the swelling will slow down. Chitosan hydrochloride at molecular weight of 227,000 and 83,000 showed different result from other gelling agents. When water was added to the cylinder chitosan hydrochloride MW. 227,000 and 83,000 were dissolved easily and became yellow clear solution within 1.5 hours.

Physical appearance of gelling agents after swelling measurement in the cylinder are presented in Table 12.

Table 12 Physical appearance of various gelling agents after complete swelling

Gelling Agents	Physical Appearance
Gelatin	Coagglulate like gel
Sodium Carboxy Methyl Cellulose	Disperse , soft
Xanthan gum	Coagglulate , hard
Pectin	Coagglulate like gel , hard
Chitosan hydrochloride MW.227,000	Yellow clear solution
Chitosan hydrochloride MW.83,000	Yellow clear solution
Chitosan glutamate MW.227,000	Swell like gel
Chitosan glutamate MW.83,000	Coagglulate

Swelling index of these gelling agents was measured by the volume occupied by gelling agents as showed in Table 13.

Table13 Swelling index of various gelling agents

Gelling Agents	Swelling Index (ml/g)
Gelatin	5.75 ± 0.66
Sodium Carboxy Methyl Cellulose	9.67 ± 0.29
Xanthan gum	6.17 ± 0.76
Pectin	6.33 ± 0.76
Chitosan hydrochloride MW.227,000	N/A*
Chitosan hydrochloride MW.83,000	N/A*
Chitosan glutamate MW.227,000	5.17 ± 0.58
Chitosan glutamate MW.83,000	Less than 4

All values were mean ± SD of three samples, * soluble solution were obtained

From Table 13 the swelling index of gelling agents were between 5.17-9.67 and could be order as follows: Sodium Carboxy Methyl Cellulose(SCMC) > Pectin > Xanthan > Gelatin > Chitosan glutamate MW.227,000. In the case of chitosan hydrochloride at both molecular weights which the salt of strong acid and well known for water solubility. At the percentage used of swelling study, soluble solutions were obtained for both molecular weight of chitosan.

In summary, it could be concluded that SCMC was the gelling agent that showed the highest swelling index.

2.3.4 Compatibility Study of Gelling Agents in Hydrophobic Base by Differential Scanning Carolimetry (DSC)

DSC scan of various gelling agents used in this experiment are presented in Figures 47 – 56.

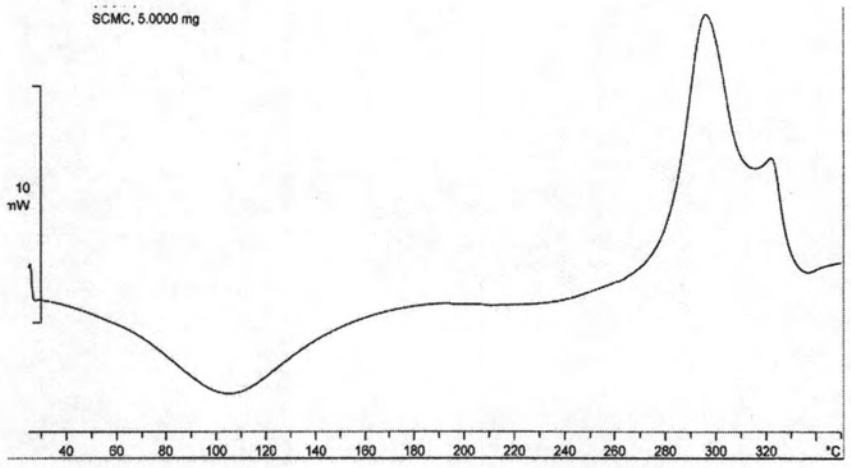


Figure 47 DSC scan of sodium carboxy methyl cellulose (SCMC)

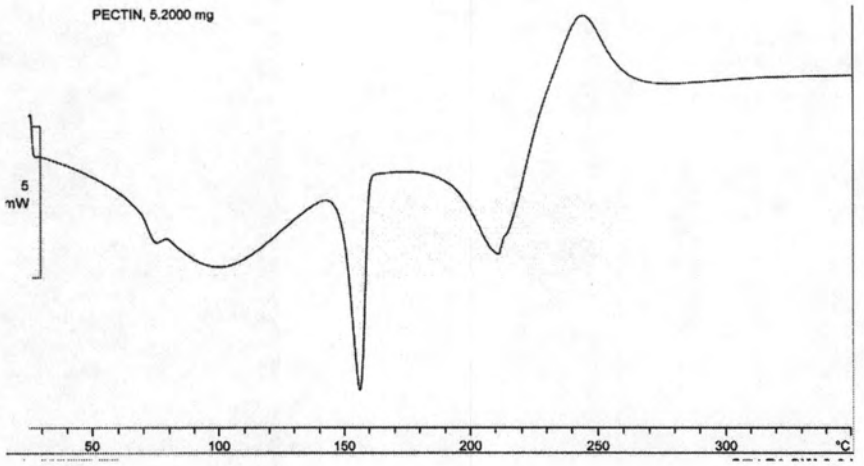


Figure 48 DSC scan of pectin

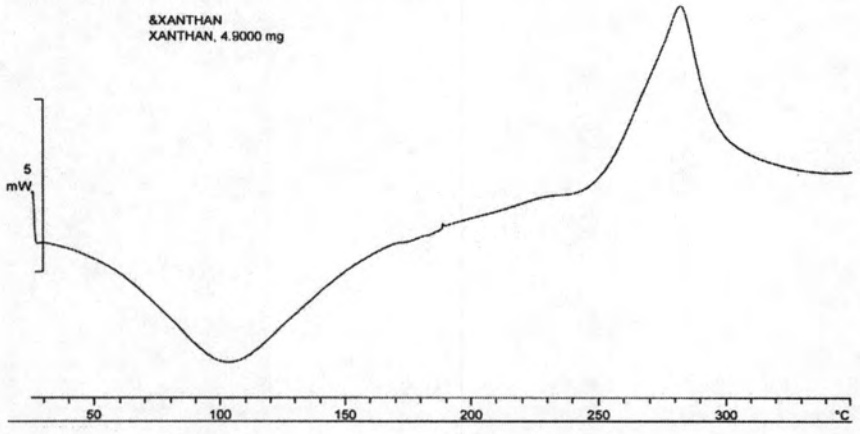


Figure 49 DSC scan of xanthan gum

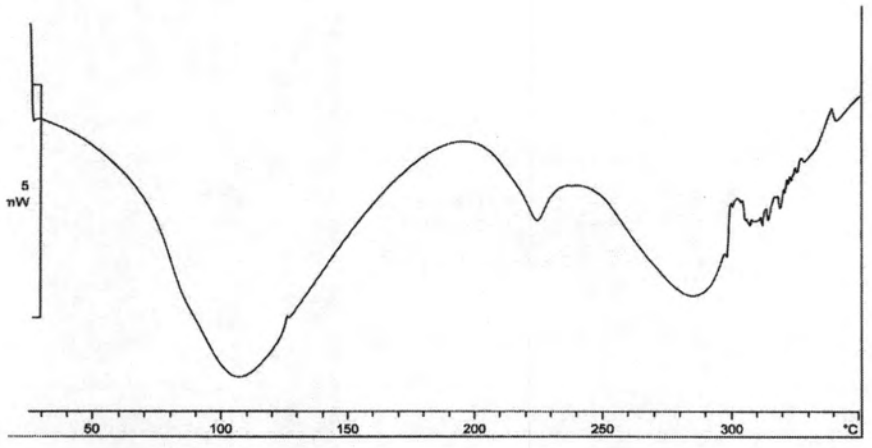


Figure 50 DSC scan of gelatin

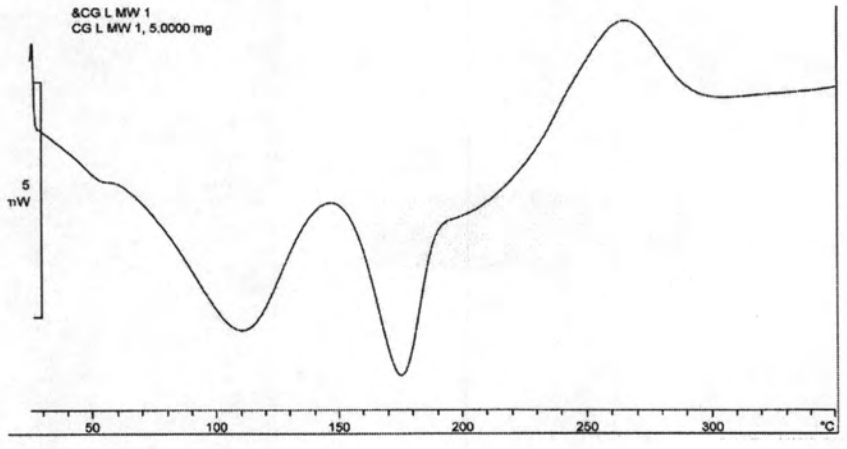


Figure 51 DSC scan of chitosan glutamate MW. 83,000

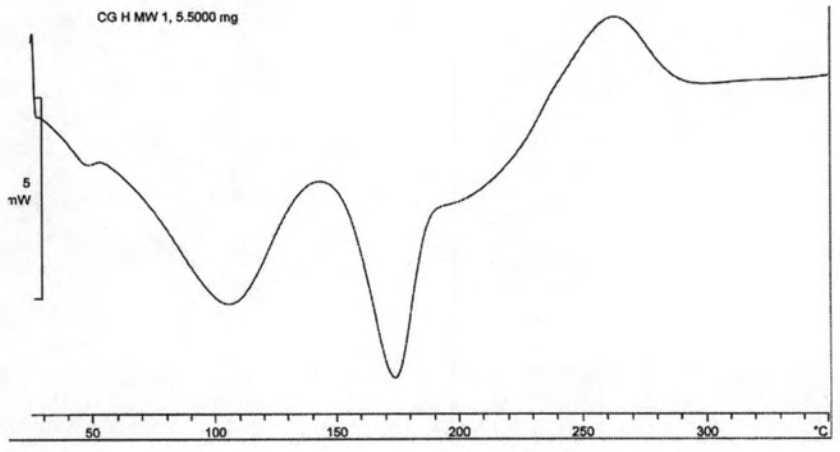


Figure 52 DSC scan of chitosan glutamate MW 227,000

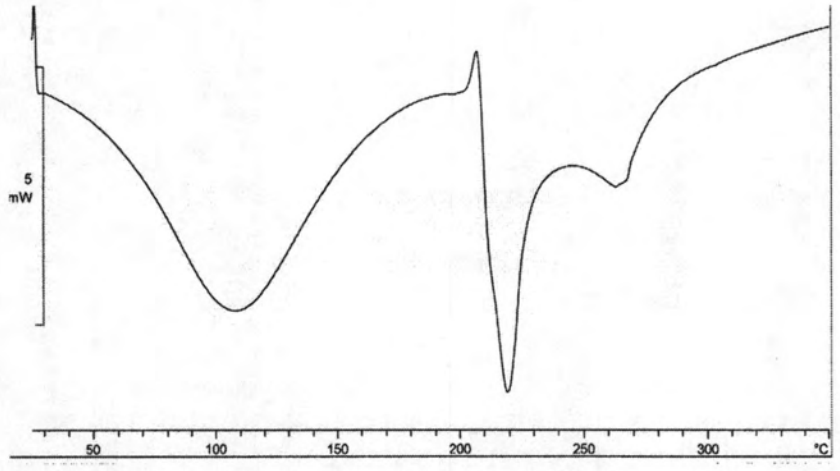


Figure 53 DSC scan of chitosan hydrochloride MW. 83,000

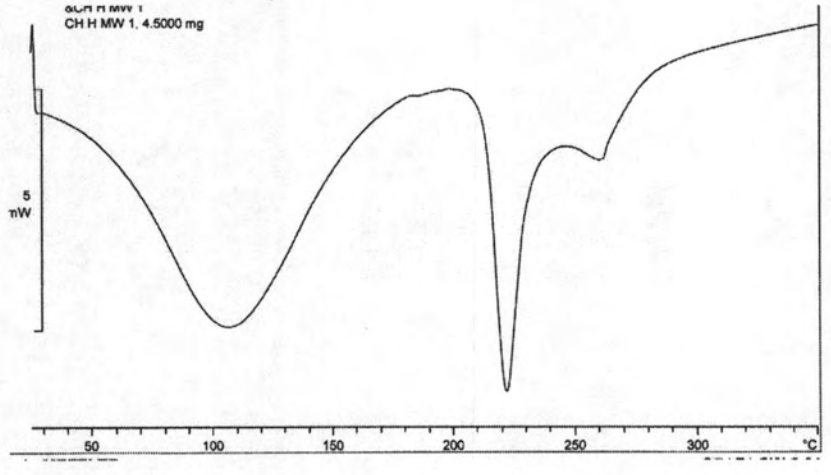


Figure 54 DSC scan of chitosan hydrochloride MW. 227,000

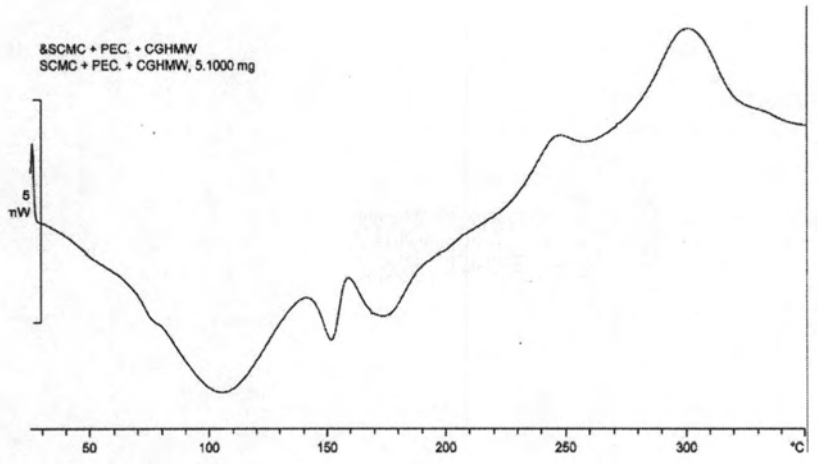


Figure 55 DSC scan of powder mixed of pectin, chitosan glutamate MW 227,000 and SCMC

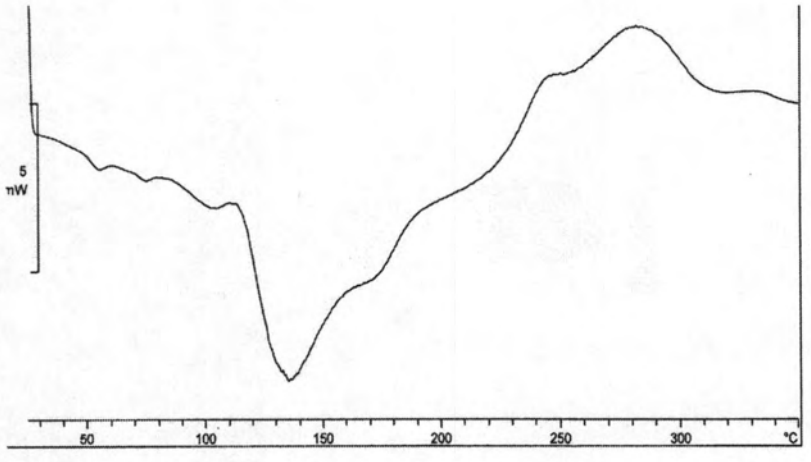


Figure 56 DSC scan of the mixture of hydrophobic base, pectin, chitosan glutamate MW 227,000 and SCMC

Figures 47-50 shows DSC scan of SCMC (cellulose polymer), pectin, xanthan gum (natural polymer) and gelatin (polypeptide), respectively. These previously polymer compounds mostly contain aliphatic hydrocarbon, hydroxyl groups, oxygen atoms except for gelatin which is linkage of various amino acid, in this case less inter-intra molecular hydrogen bond will occurred thus, leading to less crystal lattice energy and the corresponding endothermic peak from DSC scan will be broad and the onset temperature of these polymers appeared approximately the same temperature. DSC scan of chitosan salts are presented in Figures 51-54. Both chitosan hydrochloride at MW. of 227,000 and 83,000 perfomed broad endothermic peak at about 110 ° C and sharp peak at an onset temperature about 220 ° C. In the case of chitosan glutamate at MW. of 227,000 and 83,000 showed broad endothermic peak with an onset temperature at 110 ° C and sharp peak at an onset temperature about 170 ° C.

3. Determination of Active Ingredients by High Performance Liquid Chromatography Method (HPLC)

HPLC is the most suitable method to determine the active constituents of this herbal extracts both raw material and pharmaceutical dosage form because of its high sensitivity and specificity.

3.1 Mangostin

The HPLC chromatogram of mangostin obtained by adjusting mobile phase is showed in Figure 57.

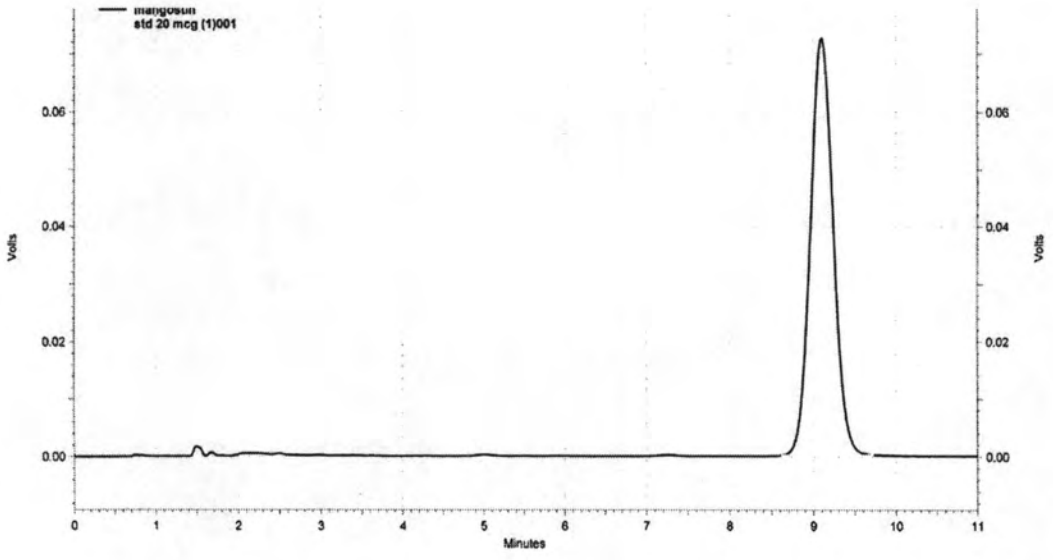


Figure 57 Typical HPLC chromatogram of standard mangostin

Validation of HPLC Method

Analytical method validation is a process to evaluate that the methods are suitable and consistent for application. The analytical parameters considered in this validation study are specificity, linearity, accuracy and precision.

Specificity

The specificity of peak was present as the resolution factor and tailing factor. The retention time and the tailing factor of mangostin were about 9.1. and 1.0, respectively. They performed the satisfactory resolution and symmetry peak.

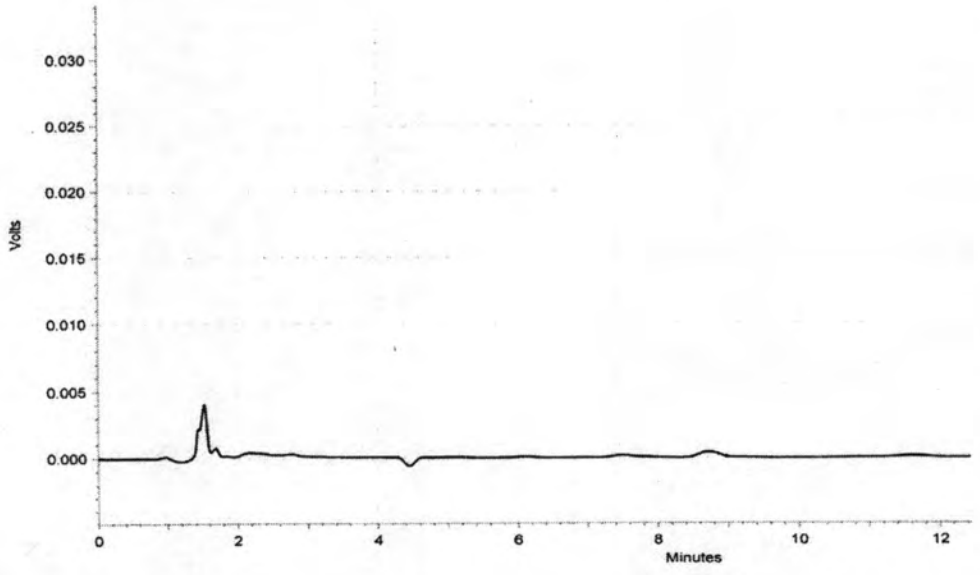


Figure 58 Typical chromatogram of oral paste formula without mangostin show only the solvent front and no interference of the other peaks are found which indicate specificity of the assay system

Linearity

A linearity study was carried out to determine whether this method could measure accurately different concentrations of mangostin. The linearity curve of peak area as a function of concentrations of standard mangostin is presented in Figure 73 in Appendix.

The standard concentration that gave linear standard curve was in the range of 5-60 µg/ml. The correlation of determination (R^2) for standard curve was about 0.9993. This result showed a good linearity of standard concentrations and peak area.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from several sampling of the same homogeneous samples. Precision of this method was expressed as the percentage of coefficient of variation (%CV) and the data are showed in Table 26 in Appendix. The % CV of mangostin was about 0.31 .The low %CV indicated the good precision of this method.

3.2 Asiaticoside

The HPLC chromatogram of mangostin obtained by adjusting mobile phase is showed in Figure 59.

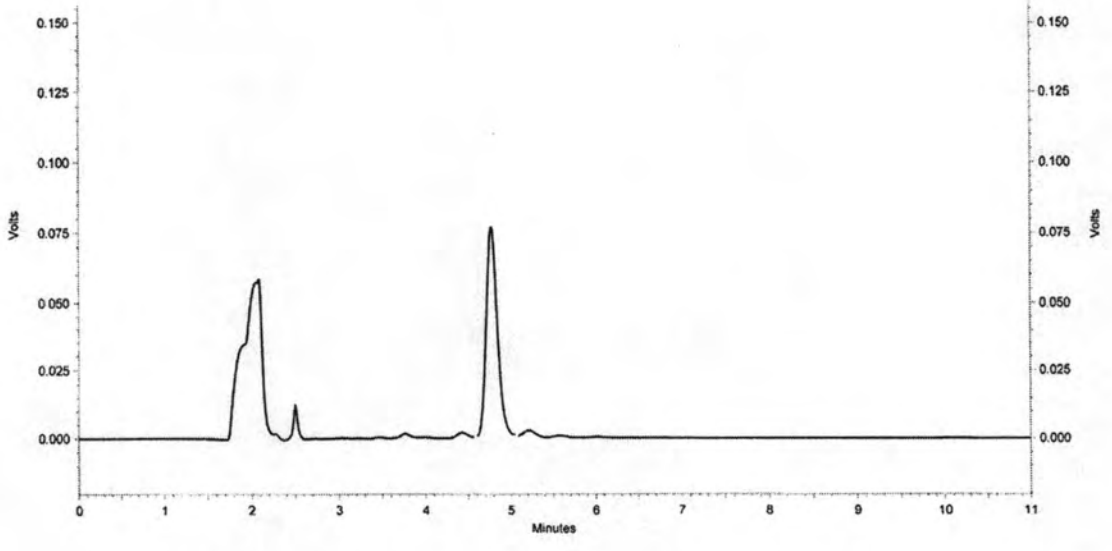


Figure 59 Typical HPLC chromatogram of standard asiaticoside

Validation of HPLC Method

Analytical method validation is a process to evaluate that the method are suitable and consistent for application. The analytical parameters considered in this validation study were specificity, linearity, accuracy and precision.

Specificity

The specificity of peak was present as the resolution factor and tailing factor and showed in Figure 59 .The retention time of asiaticoside was about 4.7. The tailing factor was 1.12. They performed the satisfactory resolution and symmetry peak.

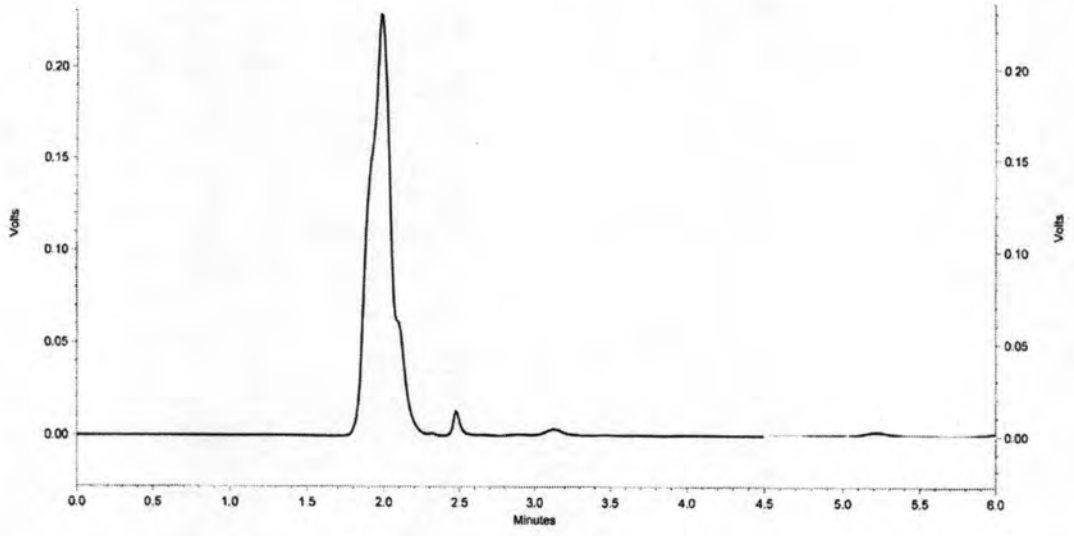


Figure 60 Typical chromatogram of oral paste formula without asiaticoside which shows only the solvent front and no interference of the other peaks are found which indicate specificity of the assay system

Linearity

A linearity study was carried out to determine whether this method could measure accurately different concentrations of asiaticoside. The linearity curve of peak area as a function of concentrations of standard asiaticoside is presented in Figure 74 in Appendix.

The standard concentration that gave linear standard curve was in the range of 50-600 μg/ml. The correlation of determination (R^2) for standard curve was about 0.9999. This result showed a good linearity of standard concentrations and peak area.

Precision

The precision of an analytical procedure expresses the closeness of agreement between a series of measurement obtained from several sampling of the same homogeneous samples. Precision of this method was expressed as the percentage of coefficient of variation (%CV) and the data are showed in Table 29 in Appendix. The % CV of mangostin was about 0.56.The low %CV indicated the good precision of this method.

4. Formulation of Herbal Extracts Oral Paste

As was mentioned earlier from the previous studied after obtained suitable lipophillic base containing 4.5 % of PE polymer. Then various gelling agents (SCMC, chitosan glutamate MW 227000 and pectin) at suitable concentration were mixed with the base and evaluated for moisture adsorption test, tensile strength, swelling index test. It was concluded that SCMC showed the greatest moisture adsorption property when compared with the others at 96 and 75 % RH. Chitosan glutamate MW.227,000 performed the highest tensile strength in tensile strength measurement. In swelling index test, pectin showed swelling index greater than other gelling agents except SCMC. Thus, from these results SCMC, chitosan glutamate MW.227,000 and pectin were chosen to formulate oral paste with hydrophobic base. In preliminary studies hydrophobic base and gelling agents were mixed together in appropriate proportion which containing 50 % of hydrophobic base and 50 % of gelling agents and testing for consumer palatable acceptability as showed in Table 14.

Table 14 Taste result of mixture of hydrophobic base and various gelling agents

Ingredients (%w/w)	Formulations															
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**	**F12**	**F13**	**F14**	**F15**	**F16**
SCMC	5	10	15	20	25	22.5	20	17.5	15	12.5	22.5	20	17.5	15	12.5	16.67
Chitosan glu. MW. 227000	22.5	20	17.5	15	12.5	5	10	15	20	25	22.5	20	17.5	15	12.5	16.66
Pectin	22.5	20	17.5	15	12.5	22.5	20	17.5	15	12.5	5	10	15	20	25	16.66
Hydrophobic base	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Taste result	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Not bitter	Not bitter	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste	Bitter taste

The results from Table 14 show that every formulation except F6 and F7 showed bitter taste after applied for oral application. These bitter tastes may result from the taste of chitosan glutamate MW.227,000. Formulas F6 and F7 were composed of chitosan glutamate MW.227,000 at 5 and 10 % respectively. It seemed that the percentage of chitosan glutamate MW.227,000 in the formulation must not exceed 10% for acceptable taste. So, the amounts of chitosan glutamate MW.227,000 in the mixture were limited to not more than 10% in the formulation of oral paste in next experiment and the results are shown in Table 15.

The results from Table 15 shows that the formulation containing chitosan glutamate MW.227,000 at 2.5 and 5 % did not adhere to mucous membrane in mouth. Ten percent of chitosan glutamate MW.227,000 in formulation showed the suitable property for both mucous adhesion and palatable taste . When oral paste F30 were applied to mucous area in the mouth it attached to tissue like a film covered mucous area and this film can stand for a long period of time.

From data obtained from Tables 14 and 15 the proportion of gelling agents which appropriate to formulate oral paste composed of 15 % of SCMC, 25% of pectin and 10 % of chitosan glutamate MW.227,000 . Thus, the oral paste formulations containing active ingredients are listed in Table 16.

Table 15 The results of an oral mucous adhesion of the mixture of hydrophobic base and various gelling agents

Ingredients (%w/w)	Formulations														
	F17	**F18**	**F19**	**F20**	**F21**	**F22**	**F23**	**F24**	**F25**	**F26**	**F27**	**F28**	**F29**	**F30**	**F31**
SCMC	37.5	32.5	27.5	22.5	17.5	35	30	25	20	15	30	25	20	15	10
Chitosan glu. MW. 227000	2.5	2.5	2.5	2.5	2.5	5	5	5	5	5	10	10	10	10	10
Pectin	10	15	20	25	30	10	15	20	25	30	10	15	20	25	30
Hydrophobic base	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
Attachment result	Not attach	Not attach	Not attach	Not attach	Not attach	Swell, Short attach	Swell, Short attach	Swell, Short attach	Swell, Short attach	Swell, short attach	Swell, attach	Swell, attach	Swell, attach	Swell, attach like film	Not swell

Table 16 Composition of oral paste containing active ingredients

Ingredients (%w/w)	Asiaticoside oral paste	Mangostin oral paste
SCMC	15	15
Pectin	25	25
Chitosan glutamate MW.227,000	10	10
Antioxidant	0.1	0.1
Asiaticoside	0.2	-
Mangostin	-	0.2
Hydrophobic base	50	50

5. An Evaluation of Oral Paste Containing Herbal Extracts

Oral paste containing herbal extracts (mangostin or asiaticoside) are packed in aluminum collapsible tubes and were studied as follows:

5.1 Physical Appearance

The physical appearance of mangostin and asiaticoside oral paste were yellowish. The color of both mangostin and asiaticoside oral paste after storage at ambient and 30 ° C were unchanged.

5.2 Assay

5.2.1 Mangostin

Validation of HPLC Method for Analyzing the Oral Paste Products of Mangostin

Specificity

An analytical method is specific whether it confirm that measured peaks is only related to the substance intended to be analyzed, targeted compound, in the presence of the extraneous components. The excipients in the formulation did not interfere with peak of active constituents. The HPLC chromatogram is showed in Figure 61.

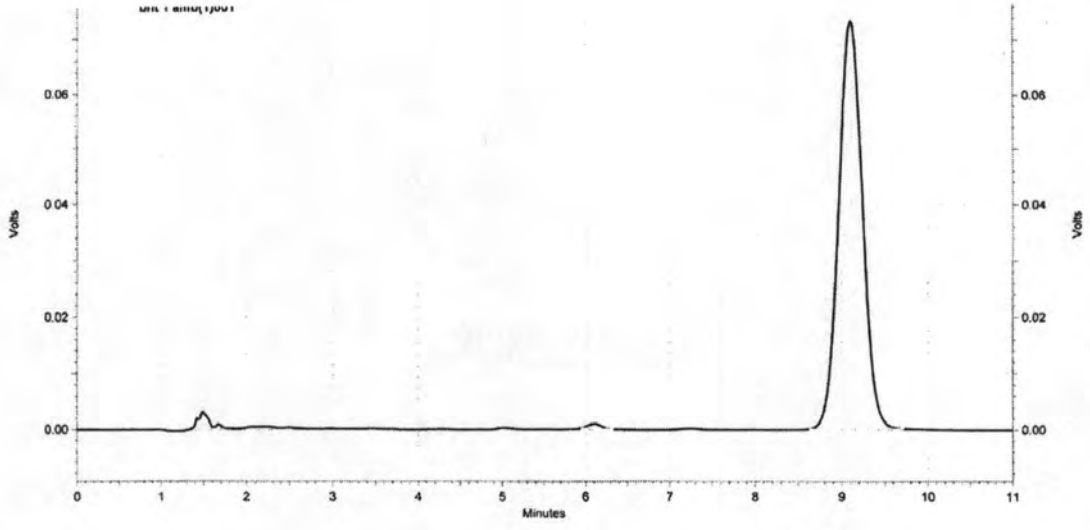


Figure 61 HPLC chromatogram of mangostin in mangostin oral paste

Accuracy

The accuracy of the purposed method defined as the percentage of the recovery, is calculated as deviation agreement between the measured value and the true value. The results are presented in Table 27 in Appendix. The ranges of the percentage of recovery were between 99.79 % - 105.67 %, thus this method claimed to be accurate for measurement of mangostin.

5.2.2 Asiaticoside

Validation of HPLC Method for Analyzing the Oral Paste Products of Asiaticoside

Specificity

An analytical method is specific whether it confirm that measured peaks is only related to the substance intended to be analyzed , targeted compound, in the presence of the extraneous components. The excipients in the formulation did not interfere with peak of active constituents. The HPLC chromatogram is showed in Figure 62.

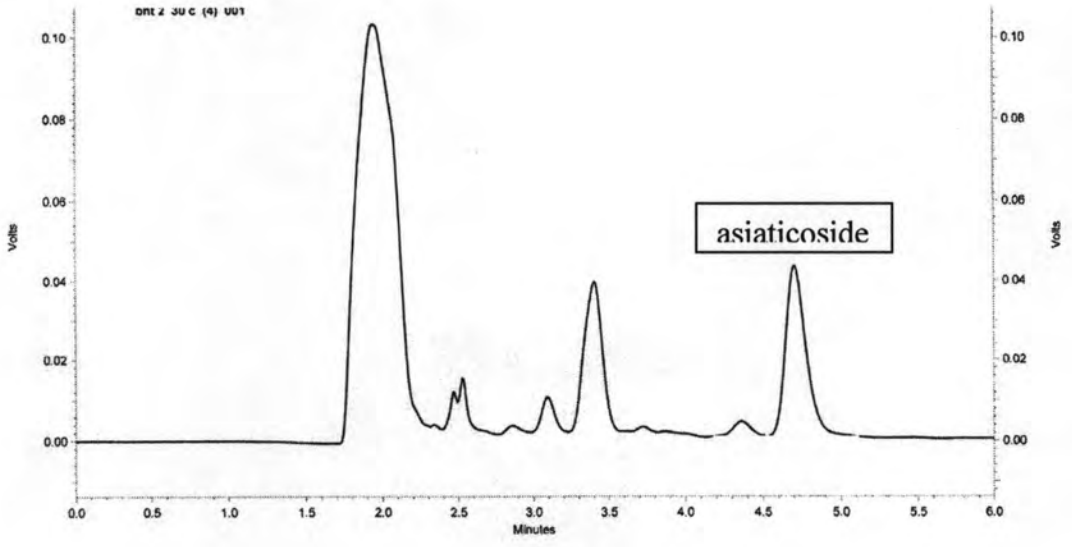


Figure 62 HPLC chromatogram of asiaticoside in asiaticoside oral paste indicate specific assay method in the presence of various components in the formula

Accuracy

The accuracy of the purposed method defined as the percentage of the recovery, is calculated as deviation agreement between the measured value and the true value. The results were presented in table 30 in Appendix. The ranges of percentage of recovery were between 99.37 % - 103.96 %, thus this method claimed to be accurate for measurement of asiaticoside.

6. Photooxidation study

6.1 Mangostin

After freshly prepared, all formulations were stored under daylight fluorescent lamp within the close cabinet. The percent contents of mangostin were analysed and are showed in Tables 17 and 18. The residual contents were calculated by comparing the corresponding value with initial as presenting in Figures 63 and 64.

Table 17 The percentage label amount of mangostin oral paste kept in tight light resistant containers.

% BHT (%w/w)	% Label amount		
	Initial	**Day 14**	**Day 28**
Blank (no BHT)	97.26	99.35	100.67
0.1	98.14	98.02	101.08
0.2	97.24	96.38	100.68
0.3	99.34	97.11	98.62

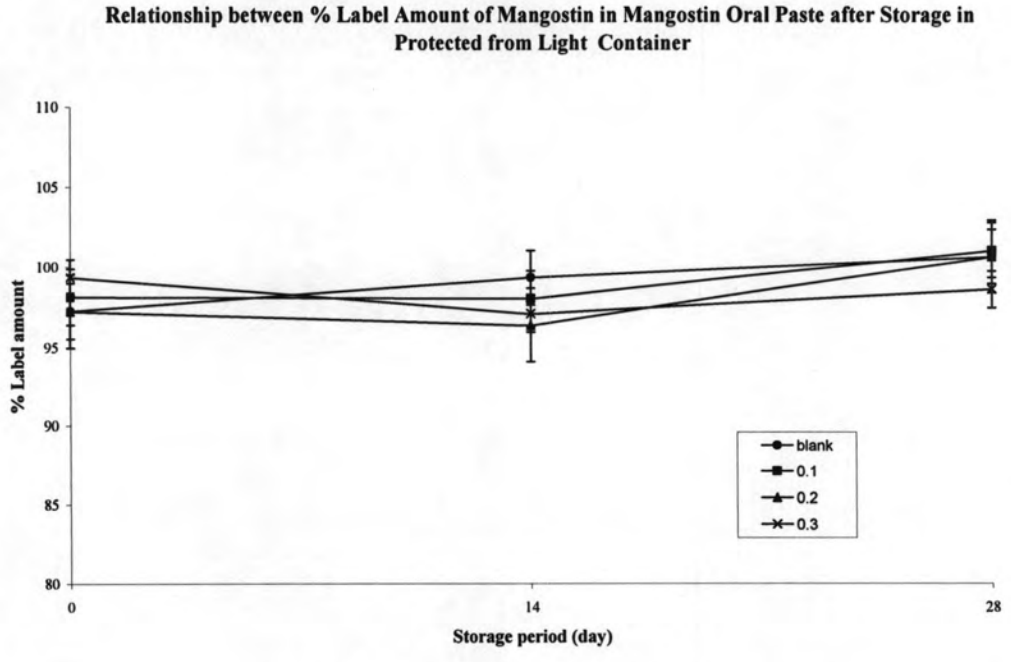


Figure 63 Storage assay values of mangostin oral paste with and without antioxidant after kept in tight light resistant containers at various time intervals (n=3)

Table 18 The percentage label amount of mangostin oral paste kept in transparent containers that light could pass through

% BHT (%w/w)	% Label amount					
	Initial	**Day 2**	**Day 5**	**Day 8**	**Day 11**	**Day 14**
0.1	96.30	91.03	93.13	92.87	93.11	93.06
0.2	100.52	98.66	99.69	98.59	100.26	98.36
0.3	99.61	99.54	100.09	99.20	101.60	101.67

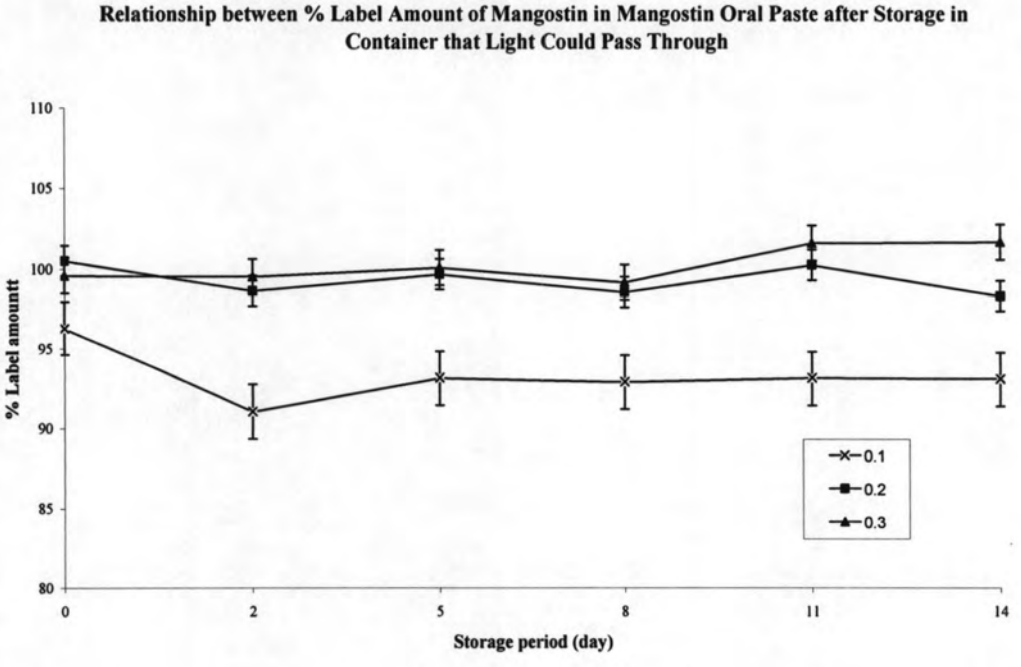


Figure 64 Storage assay values of mangostin oral paste containing various amounts of antioxidant after kept in containers that light could pass through at various time intervals (n=3)

From Tables 17 and 18, the percent contents of oral paste containing mangostin kept in containers protected from light seem to be unchanged at various storage time intervals and the assay values were fluctuated within acceptable limit of the experiment error. In the transparent containers that light could pass through percent contents of mangostin in oral paste were slightly decreased in formulation containing 0.1 and 0.2% BHT but at the level of 0.3% antioxidant percent contents of mangostin in oral paste seem to be unchanged. From these results it could be concluded that mangostin is sensitive to light and the formulation containing antioxidant like BHT at 0.3 % could be protect active ingredient from photoxidation reaction.

6.2 Asiaticoside

The percent contents of asiaticoside are presented in Tables 19 and 20. The residual contents were calculated by comparing the corresponding value with initial as presenting in Figures 65 and 66.

Table 19 The percentage label amount of asiaticoside oral paste with and without antioxidant kept in container which protected from light

% BHT (%w/w)	% Label amount		
	Initial	**Day 14**	**Day 28**
Blank (no BHT)	95.28	97.40	97.20
0.1	103.12	104.21	103.74
0.2	105.16	103.96	102.70
0.3	105.78	103.07	104.88

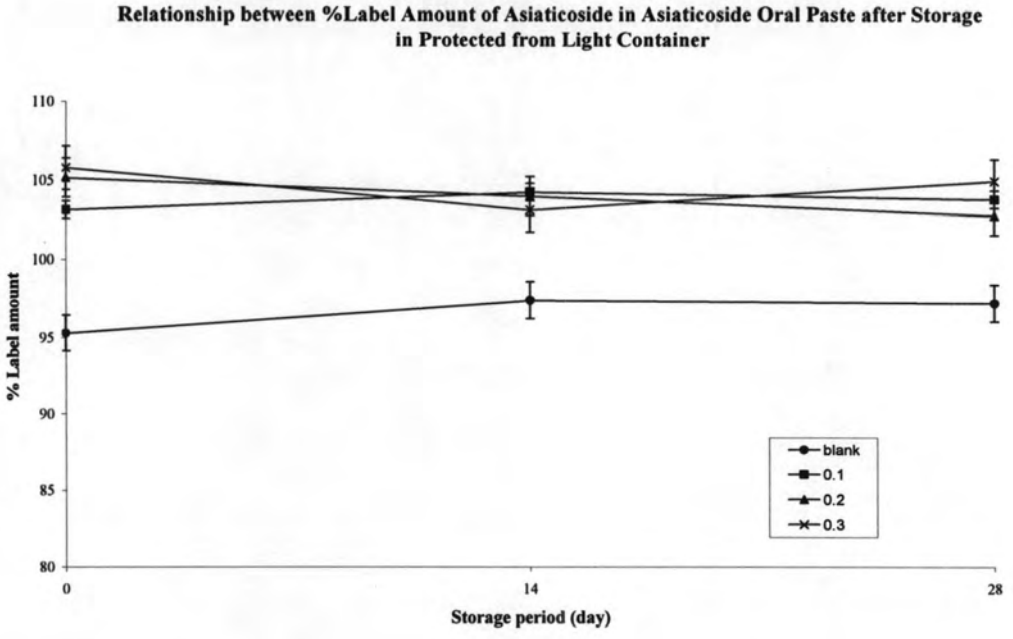


Figure 65 Storage assay values of asiaticoside oral paste with and without antioxidant after kept in container that protected from light at various time intervals (n=3)

Table 20 The percentage label amount of asiaticoside oral paste kept in transparent container that light could pass through

% BHT (%w/w)	% Label amount					
	Initial	**Day 2**	**Day 5**	**Day 8**	**Day 11**	**Day 14**
0.1	98.04	100.57	99.46	98.88	102.47	99.72
0.2	99.38	100.92	102.85	101.66	99.91	100.27
0.3	105.20	102.60	103.64	104.49	103.35	103.65

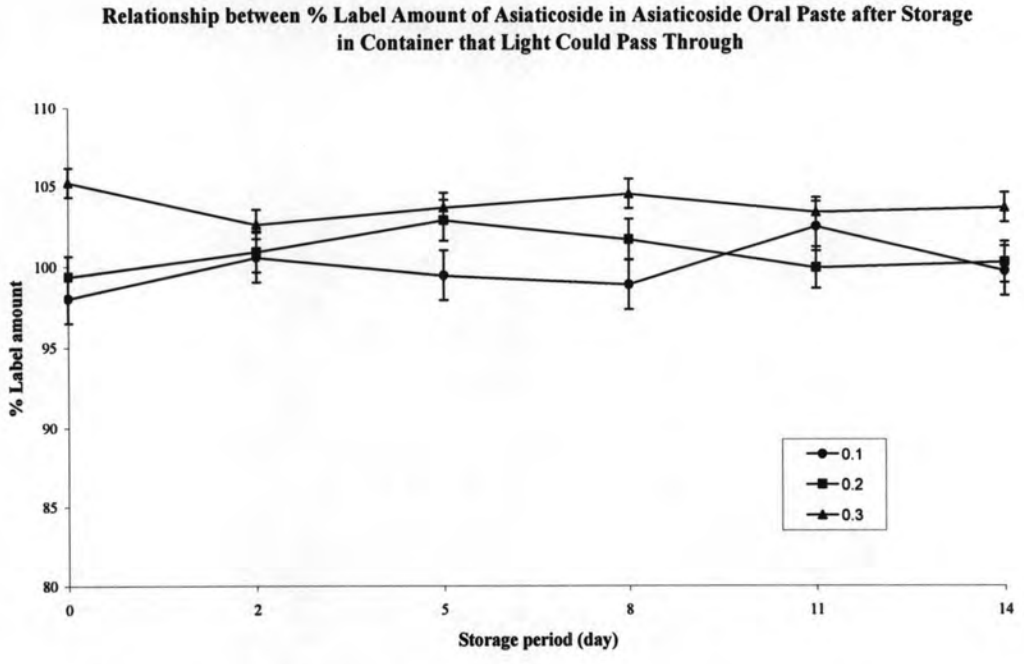


Figure 66 Storage assay values of asiaticoside oral paste containing various amounts of antioxidant after kept in the transparent container that light could pass through (n=3)

Data obtained from Table 19 shows the percent contents of asiaticoside oral paste kept in containers protected from light seem to be unchanged at various storage time intervals and the assay values were fluctuated within acceptable limit of experimental error. Table 20 shows that the percent content of asiaticoside were slightly increased in formulation containing 0.1 and 0.2 % antioxidant but in level of 0.3 % BHT the percent content of asiaticoside were decreased and the assay values were fluctuated within acceptable limit of experimental error.

Data from Tables 17-20 and Figures 63-66 shows that the percent contents of both mangostin and asiaticoside in formulation were slightly changed within acceptable limit of not more than ± 5%. These results indicated that oxidation reaction could not degrade the both active ingredients (mangostin and asiaticoside) in formulations using BHT as antioxidant.

Structures of mangostin and asiaticoside indicate that functional moieties contained in the structure of the drug may be sensitive to oxidation reaction. For oxidation reaction, oxygen, heavy metals and light are the main factors that initiate degradation of the active compounds. So, if the formulations were protected from these factors the oxidation reaction could be prevented. Further more for lipophillic base water was not used in these formulations thus oxidation reaction from oxygen was not occurred. In addition, aluminum collapsible tubes used in this study were coated with epoxy on surface which direct contact to the formulation to protect from heavy metals. BHT used as antioxidant could dissolve in mineral oil which is one of compositions of hydrophobic base. Therefore, it could protect the formulation from both heavy metals and photooxidation.

7. Stability Study

In preliminary studies about stability of oral paste formulations containing herbal extracts it was found that the physical appearance of formulations that stored under accelerated condition (45 ± 2° C, 75 ±5 %RH) were changed. So, the oral paste formulations were stored under 30 ± 2 ° C instead of the previous accelerated condition. The hydrolysis reaction was not occurred because there was no water contain in the formulations. The possible factor that influent the change of color of the formulation may be oxidation especially photoxidation reaction due to the functional moieties of mangostin (i.e. phenolic OH, ether, conjugated double bond, etc.) and asiaticoside (i.e. ether, chiral center etc.). Therefore, BHT was added in the formulations for stability study. Stability of mangostin and asiaticoside in oral paste were evaluated as follows:

7.1 Mangostin

Percent remaining for mangostin was assay using HPLC. The calibration curves of the drug were constructed as previously mentioned. For mangostin which containing phenolic OH, ether groups and conjugated double bond in the chemical structure, thus will leading to oxidation reaction. In this case, BHT at suitable concentration will be required in the formulation.

After freshly prepared, all formulations of oral paste containing mangostin were divided into 2 groups; the former group was the mangostin oral paste formulations containing BHT 0.1- 0.3 % which stored at ambient condition (25 ± 2° C) and the latter group was the mangostin oral paste formulations containing BHT 0.1- 0.3 % which stored at 30 ± 2° C. The percent contents of mangostin were analyzed and are showed in Tables 21-22. The storage assay values of mangostin oral paste at various time intervals are showed in Figure 67.

Table 21 The percentage label amount of mangostin in mangostin oral paste at various amounts of BHT after storage at ambient temperature (25 ± 2°C) for 4 months

% BHT (%w/w)	% Label amount					
	initial	2 weeks	1 month	2 months	3 months	4 months
0.1	99.21	99.96	99.20	99.34	98.38	99.45
0.2	100.09	103.22	99.69	103.5	99.24	100.32
0.3	104.61	104.31	102.72	106.20	101.05	102.74

Table 22 The percentage label amount of mangostin in mangostin oral paste at various amounts of BHT after storage at 30 ° C for 4 months

% BHT (%w/w)	% Label amount					
	initial	2 weeks	1 month	2 months	3 months	4 months
0.1	98.55	102.60	99.48	101.81	98.84	102.12
0.2	101.67	104.08	102.09	103.15	101.44	103.10
0.3	104.53	107.44	105.98	107.87	107.12	105.91

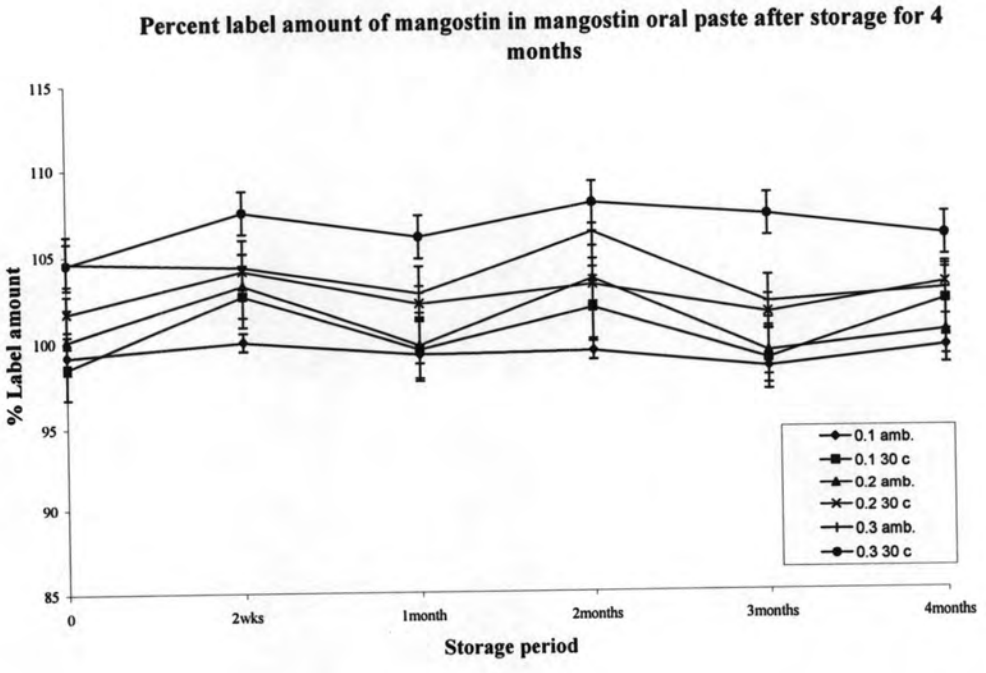


Figure 67 Storage assay values of mangostin oral paste containing various amounts of BHT after storage at ambient temperature and 30 ° C for 4 months (n=3)

Data obtained from Tables 21 and 22 the percent contents of oral paste containing mangostin in every level of antioxidants seem to be unchanged for both storage at ambient temperature and 30 ° C conditions and the assay values were fluctuated within acceptable limit of experimental error. In addition, the physical appearance of the formulation after passed 4 months at ambient and 30 ° C conditions looked like at initial condition. It could be concluded that mangostin oral paste using BHT as antioxidant in this study passed the stability studies both an ambient and accelerated conditions.

7.2 Asiaticoside

Percent remaining for asiaticoside was assay using HPLC. The calibration curves of the drug were constructed as previously mentioned. For asiaticoside which containing chiral center and ether groups in the molecule may lead to photoxidation. In this case, BHT will be required in the formulation.

In this study, asiaticoside oral pastes were divided into 2 groups; the former group was the asiaticoside oral paste formulations containing BHT 0.1- 0.2 % which stored at ambient condition (25 ± 2° C) and the latter group was the asiaticoside oral paste formulations containing BHT 0.1- 0.2 % which stored at 30 ± 2° C. The percent contents of asiaticoside were analyzed as showed in Tables 23-24. The storage assay values of asiaticoside oral paste at various time intervals are showed in Figure 68.

Table 23 The percentage label amount of asiaticoside in asiaticoside oral paste containing BHT 0.1 and 0.2 percent after storage at ambient temperature (25 ± 2°C) for 4 months

% BHT (%w/w)	% Label amount					
	initial	2 weeks	1 month	2 months	3 months	4 months
0.1	112.16	110.59	112.58	113.76	116.09	114.96
0.2	99.44	100.08	102.77	101.26	102.78	101.11

Table 24 The percentage label amount of asiaticoside in asiaticoside oral paste containing BHT 0.1 and 0.2 percent after storage at 30 ° C for 4 months

% BHT (%w/w)	% Label amount					
	initial	2 weeks	1 month	2 months	3 months	4 months
0.1	107.24	107.97	106.88	108.92	108.86	109.85
0.2	98.80	102.77	101.14	100.16	102.21	100.60

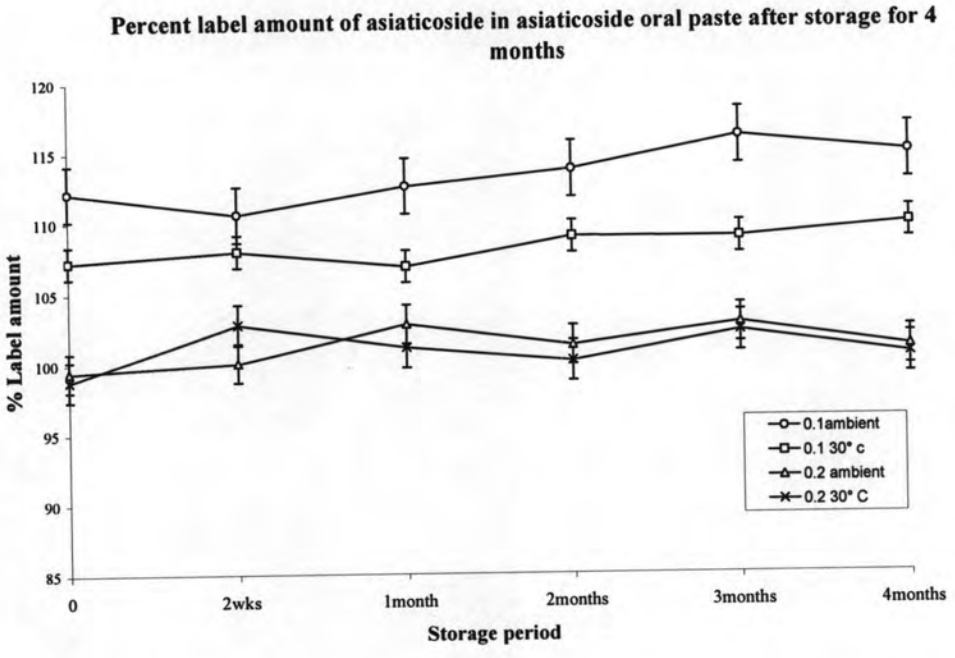


Figure 68 Storage assay values of asiaticoside oral paste containing various amounts of BHT after storage at ambient temperature and 30 ° C for 4 months (n=3)

The results from Tables 23 and 24 shows that the percent contents of oral paste containing asiaticoside in every level of antioxidants seem to be unchanged for both at ambient temperature and 30 ° C conditions and the assay values were fluctuated within acceptable limit of experimental error.

Data from Tables 21-24 and Figures 67-68 shows that the percent contents of both mangostin and asiaticoside in formulation were slightly changed within acceptable limit of not more than 5%. These results indicated that active ingredient (mangostin and asiaticoside) in formulation are not degrade in stability study both ambient and accelerated conditions.

Figures 69-72 shows DSC scan of mangostin, asiaticoside, mangostin oral paste formulation and asiaticoside oral paste formulation, respectively.

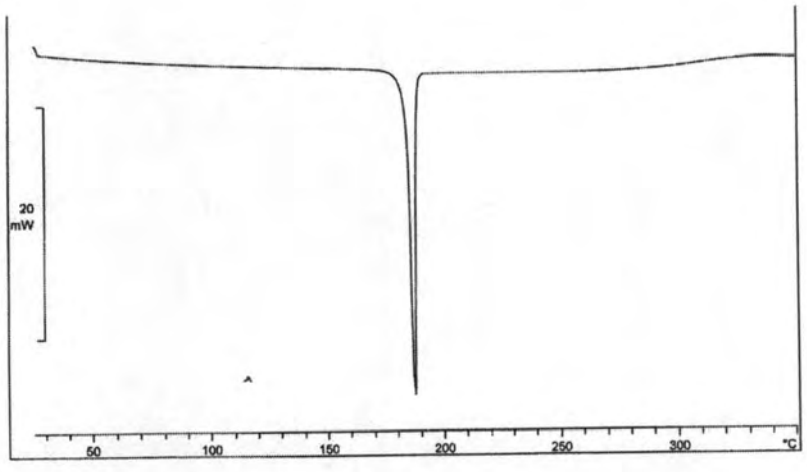


Figure 69 Typical DSC scan of mangostin extracted from *Garcinia mangostana*

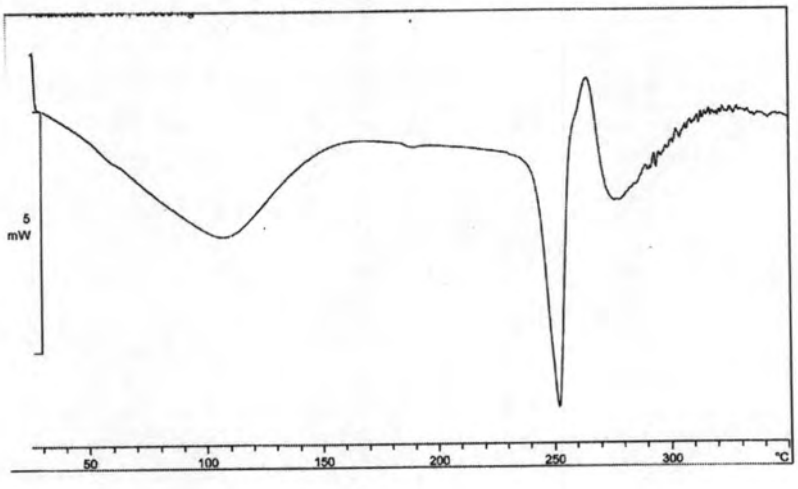


Figure 70 Typical DSC scan of asiaticoside extracted from *Centella asiatica*

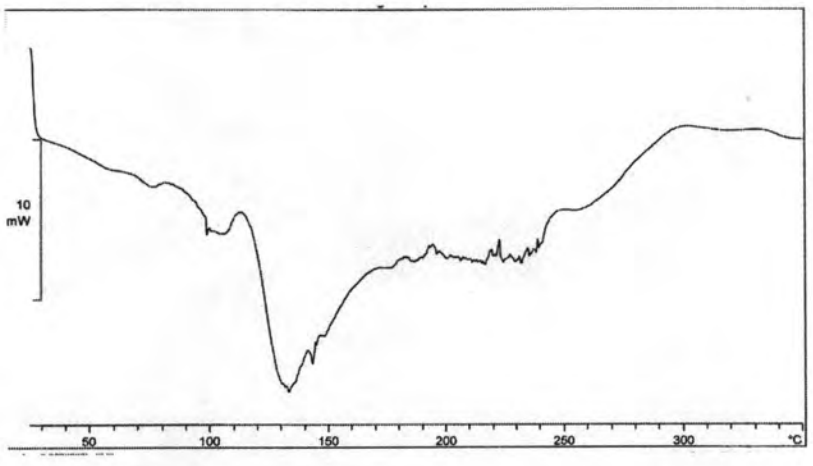


Figure 71 DSC scan of mangostin oral paste formulation

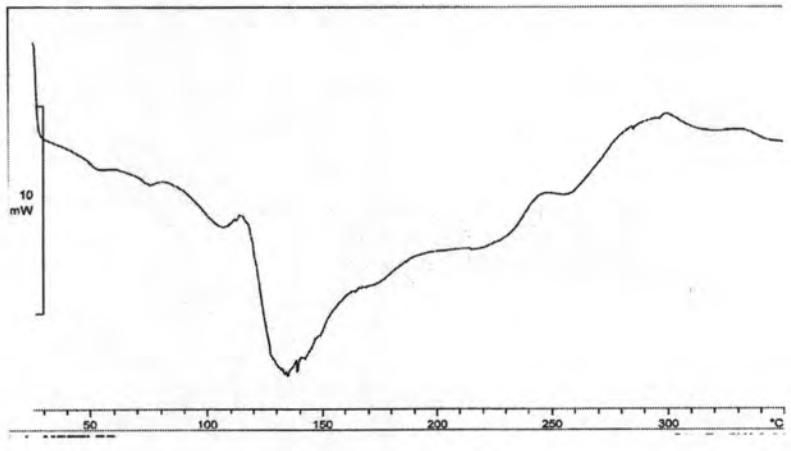


Figure 72 DSC scan of asiaticoside oral paste formulation

From Figure 69 typical DSC scan of mangostin shows sharp endothermic peak at about 180 ° C. It may indicate relationship between molecular structure of mangostin and DSC chromatogram. Structure of mangostin shows many phenolic OH groups in the molecule. A lot of phenolic OH in molecule may promote the hydrogen bonding both inter and intramolecular hydrogen bonding between and in molecule of active drug. This phenomena may make the molecule distort and close together until it reach an appropriate position. The lattice structure of this molecule must use more energy to break (i.e. high crystal lattice energy). So, the sharp endothermic peak was occurred. From Figure 70 typical DSC scan of asiaticoside

shows broad endothermic peak at 100 ° C and sharp endothermic peak at 250 ° C. According to the main chemical moiety in the core structure of asiaticoside composes of five hydrocarbon ring and various hydroxyl groups, these could enhance the molecular lattice crystal and also packing of the asiaticoside.

When mangostin was incorporate into oral paste DSC scan of mangostin oral paste was different from mangostin alone. Figure 71 shows DSC scan of mangostin oral paste formulation that the large endothermic peak was occurred at about 140 ° C which was similar to DSC scan of mixture of hydrophobic base, pectin, chitosan glutamate MW.227000 and SCMC as previously described. Like mangostin oral paste formulation, DSC scan of asiaticoside oral paste formulation in Figure 72 also shows the large endothermic peak at 140 ° C too. These phenomenon results from the influence of various polymers in the formula which mainly compose of long chain aliphatic hydrocarbon, in this case less polariability of the system will occurred and result in broad endothermic from DSC scan.