CHAPTER IV

RESULTS AND DISCUSSION

1. Glibenclamide

1.1 Characterization of raw material glibenclamide.

Glibenclamide samples were initially characterized using X-ray powder diffraction (XRPD) and differential scanning calorimetry (DSC).

1.1.1 X-ray powder diffraction (XRPD)

The X-ray diffraction pattern of glibenclamide as initially received shows strong and sharp peaks. Figure 13 shows that both the intensities and positions of the peaks are identified as form I of crystalline glibenclamide. (Suleiman and Najib, 1989)

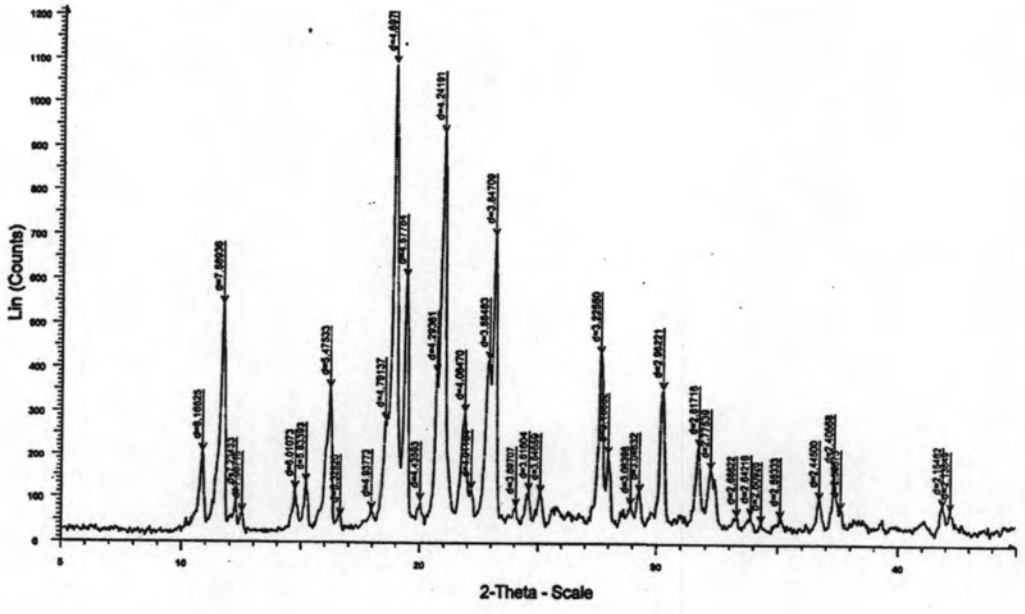

Figure 13 X-ray powder diffractogram of raw material glibenclamide.

1.1.2 Differential scanning calorimetric (DSC)

The DSC thermogram of the raw material glibenclamide (Figure 14) shows one endothermic peak at 174.87 °C corresponding to the melting point of form I crystalline glibenclamide. (Suleiman and Najib, 1989)

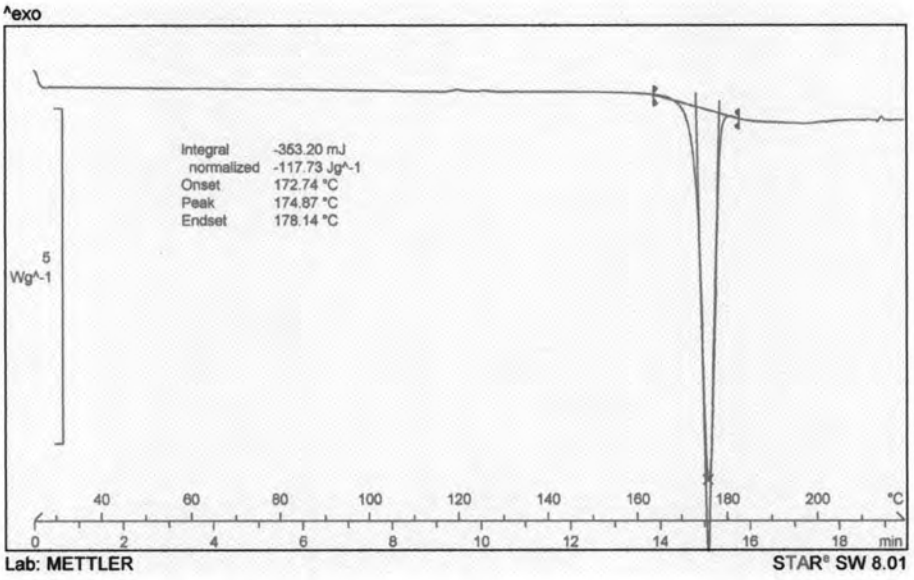

Figure 14 DSC thermogram of raw material glibenclamide.

1.1.3 High performance liquid chromatography (HPLC)

Analytical 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 linearity, precision and specificity.

Linearity

The linear equation of the curve obtained by plotting the peak area at each level prepared versus the concentrations of each standard is shown in Figure 38 and Table 13 (Appendix A). The standard concentration that gave linear standard

curve was in the range of 4.5 to 33.6μg/ml. The regression coefficient (R^2) for standard curve was 0.9997. This resulted showed a good linearity of peak area and standard concentration.

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. Precision of the method was expressed as the percentage of relative standard deviation (%RSD) and the data were shown in table 14 (Appendix A). The %RSD of glibenclamide solution on 3 days was in the range of 0.785 to 1.160. The low %RSD indicated the good precision of this method.

Specificity

The resolution between impurity and glibenclamide peak was 21.53. Tailing factor of impurity peak was 1.155 and glibenclamide was 0.966. The resolution (>1.5) and tailing factor (<2.0) indicated the good system suitability of this method as shown in Table 15(Appendix A).

1.2 Preparation of Amorphous glibenclamide powder.

In this study, amorphous glibenclamide powder was prepared by melting and quench cooling techniques. Melting and quench cooling of crystalline glibenclamide was expected to result in completely amorphous product. The obtained solidified melt of glibenclamide was transparent glassy and brittle. The X-ray diffraction pattern (Figure 15) of glibenclamide after melting and quench cooling produced weak and diffused diffraction spectra characteristic of amorphous material. Furthermore, DSC thermogram of solidified melt (Figure 16) shows one broad peak approximately 70°C. The endothermic peak appeared to be due to glass transition of amorphous form. These results further confirmed that glibenclamide after melting and quench cooling technique resulted in an amorphous nature.

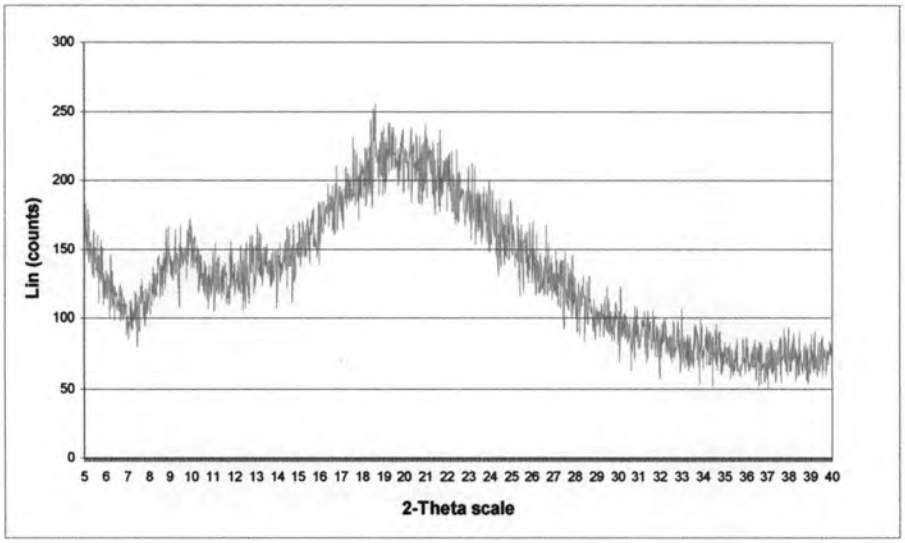

Figure 15 X-ray powder diffractogram of glibenclamide after melting and quench cooling.

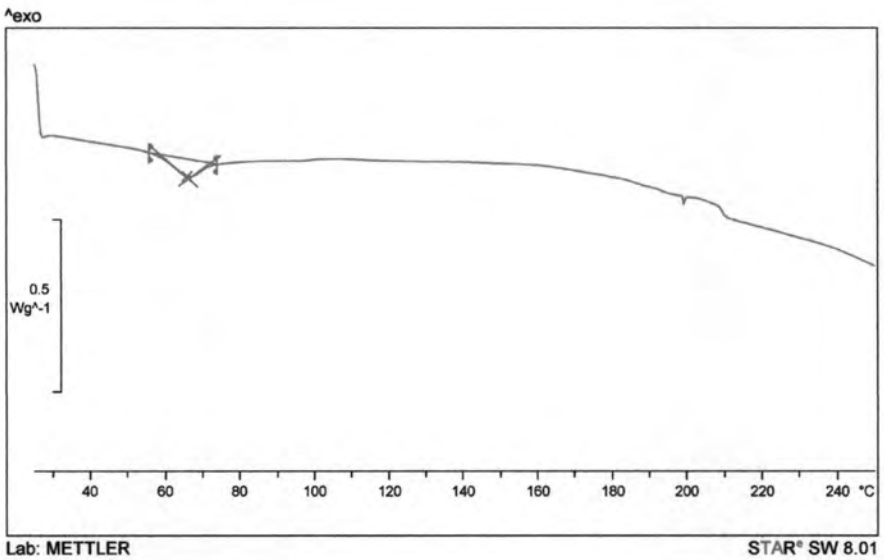

Figure 16 DSC thermogram of glibenclamide after melting and quench cooling.

1.3 Preparation of amorphous glibenclamide tablet

The amorphous glibenclamide powder 350 mg were place in ½ inch diameter die and pressure of 500 psi was introduced for 2 minutes to compact the tablets. The compression force of 500 psi did not affect the polymorphic

transformation of amorphous glibenclamide. DSC thermograms in Figure 17 showed that the amorphous glibenclamide remained unchange after compression.

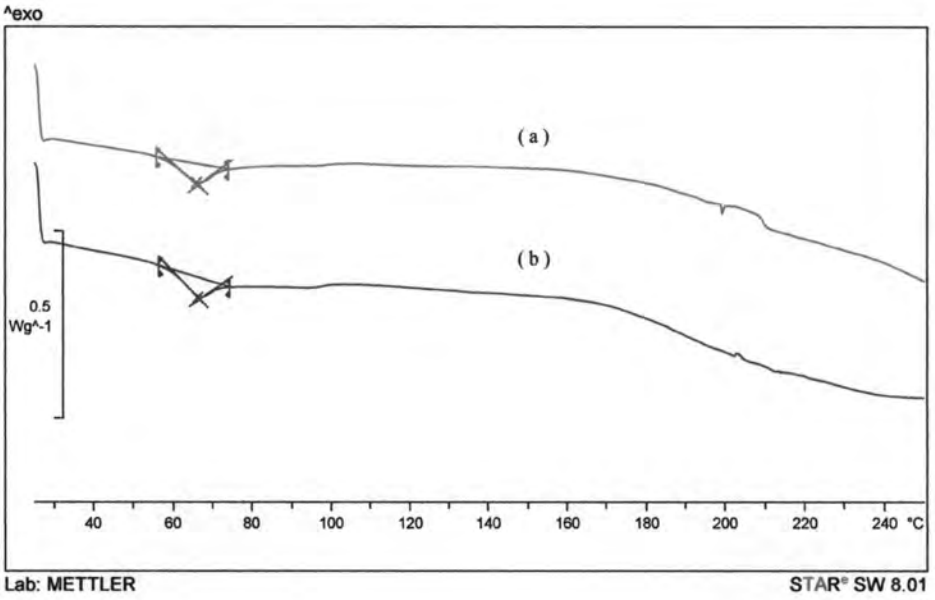

Figure 17 DSC thermograms of amorphous glibenclamide: amorphous glibenclamide powder (a), amorphous glibenclamide tablet after compression (b)

1.4 Stability of amorphous glibenclamide powder and tablets

To investigate the temperature effect on stability of the amorphous glibenclamide, solidified melt glibenclamide were placed at various elevated temperature in a closed container for 4 week. Every one week the samples were collected for analysis.

1.4.1 X-ray powder diffraction (XRPD)

The X-ray diffraction pattern of amorphous glibenclamide after storage at temperatures 60°C and 80°C for 2 weeks retained weak and diffused diffraction pattern (Figure 18). There were showed the intensity in the diffraction patterns were little change when increased storage temperature. Samples still

displayed an amorphous nature. It can be noted that X-ray diffraction data did not detect any other polymorphic forms of amorphous glibenclamide after storage.

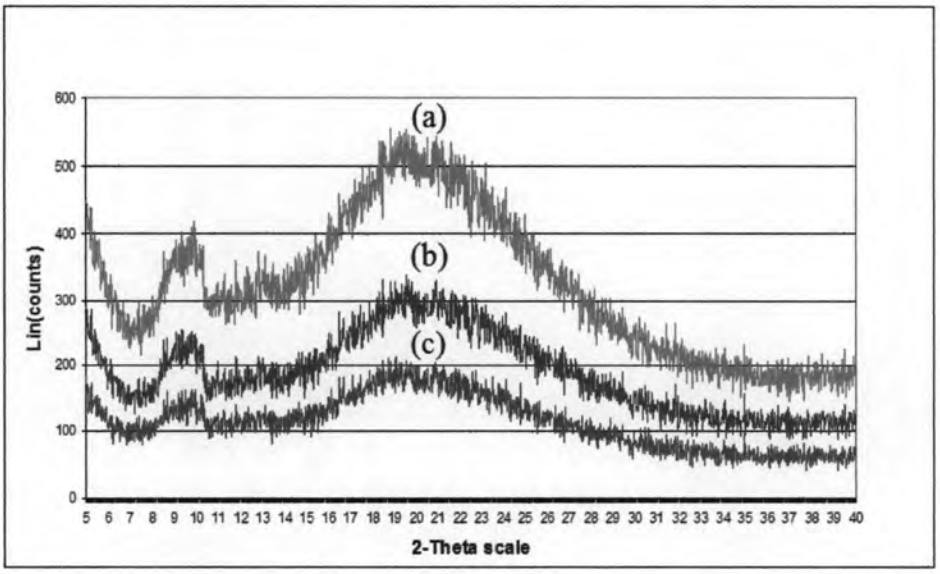

Figure 18 X-ray powder diffractograms of initial amorphous glibenclamide powder (a) amorphous glibenclamide after storage at different temperatures for 2weeks at 60°C (b) and 80°C(c)

1.4.2 Differential scanning calorimetry (DSC)

The physical aging of amorphous glibenclamide was determined by differential scanning calorimetry (DSC). The effects of aging on the DSC curve of amorphous glibenclamide are shown in Figure 19. This Figure indicates the partial transformation of amorphous form to the crystalline form upon storage at 50°C, 60°C and 80°C after 2 weeks.

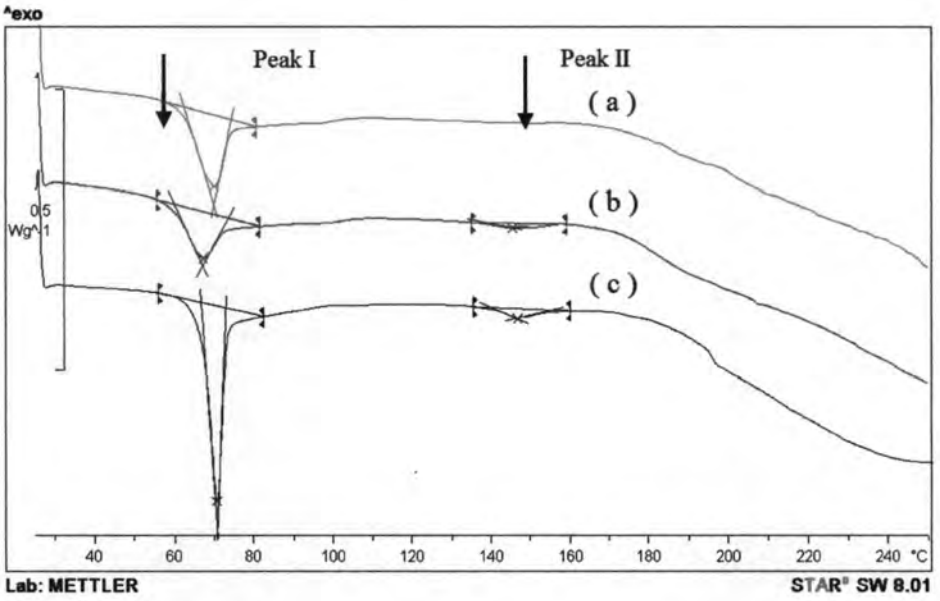

Figure 19 DSC Thermograms of amorphous glibenclamide powder after storage at different temperatures for 2 weeks: 50°C (a), 60°C (b) and 80°C (c).

Figure 19 shows the stability results of amorphous glibenclamide powder which was kept at 50°C, 60°C and 80°C for 2 weeks. The thermograms of amorphous glibenclamide at 50°C showed only one endothermic peak at 70°C while, at 60°C and 80°C, there were 2 peaks at approximately 70°C and 150°C. A glass transformation occurred at approximately 70°C a subsequent melting of the minute crystalline from produce during storage occurred at 150°C.

Therefore, the further experiment was studied the effects of storage time and temperature on powder and tablets of glibenclamide. The samples were kept at 50°C and 60°C for 4 weeks. Over a period of every one week, the samples were collected for evaluation using DSC.

Figures 20 and 21 show the thermograms of amorphous glibenclamide powder and tablets which had been kept at 50°C for 4 weeks. All of thermograms gave only endothermic peak at 70°C. In addition, the endothermic energy (J/g) which is shown in Table 3 of amorphous glibenclamide powder and tablets increased slightly when the storage time was extended.

Figures 22 and 23 display the thermograms of amorphous glibenclamide powder and tablets which had been kept at 60°C for 4 weeks. The thermograms show two endothermic peaks at 70°C and 150°C when amorphous glibenclamide powder and tablets had been kept after 2 weeks. Then, the endothermic peak at 150°C was clear when the storage time increased. Similarly, the endothermic energy (J/g), which is shown in Table 4, increased when the storage time was extended for both peaks.

A comparative study of the different temperatures presented in amorphous and partially crystallized glibenclamide. DSC measurements of these samples showed the appearance of a small endothermic peak designated as "Peak II". This peak increases and shifts towards higher temperatures as the sample is further thermally treated. Among various conditions, temperature provides an approach to induce phase transformation between metastable structures; solid-state amorphization and subsequent recrystallization, has been induced by temperature above the glass transition temperature.

Although, energy absorption of DSC results was varied and was difficult to define a stage of transformation, it could be used to predict a decrease in solubility of amorphous glibenclamide when partial crystalline solid was initiated.

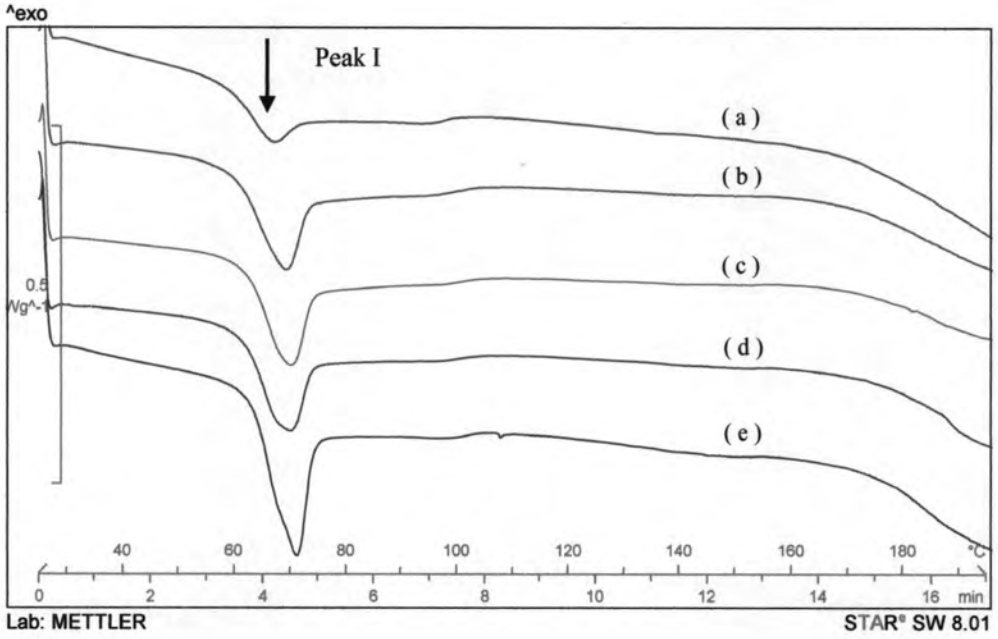

Figure 20 DSC thermograms of amorphous glibenclamide powder after storage at 50°C; amorphous glibenclamide after melt quenching (a),1 weeks (b), 2 weeks (c), 3 weeks(d) and 4 weeks(e).

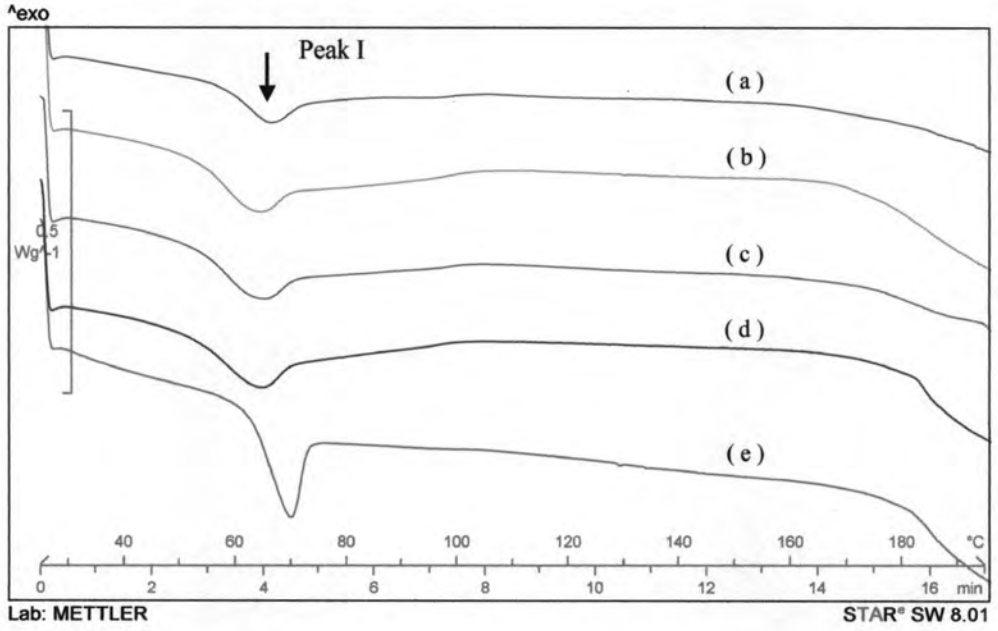

Figure 21 DSC thermograms of amorphous glibenclamide tablets after storage at 50°C ; amorphous glibenclamide after melt quenching (a),1 weeks (b), 2 weeks (c), 3 weeks(d) and 4 weeks(e).

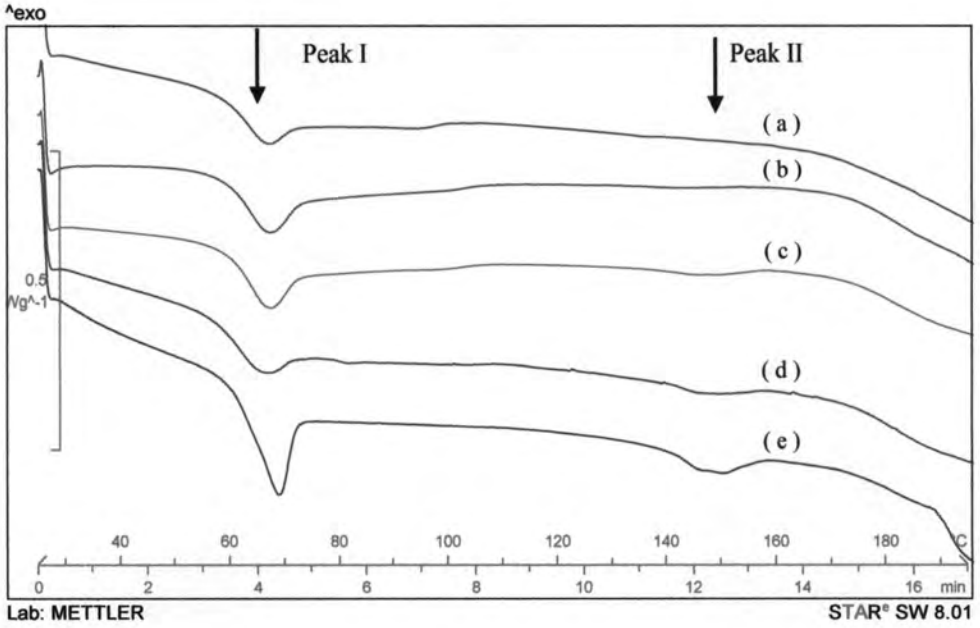

Figure 22 DSC thermograms of amorphous glibenclamide powder after storage at 60°C; amorphous glibenclamide after melt quenching (a),1 weeks (b), 2 weeks (c), 3 weeks(d) and 4 weeks(e).

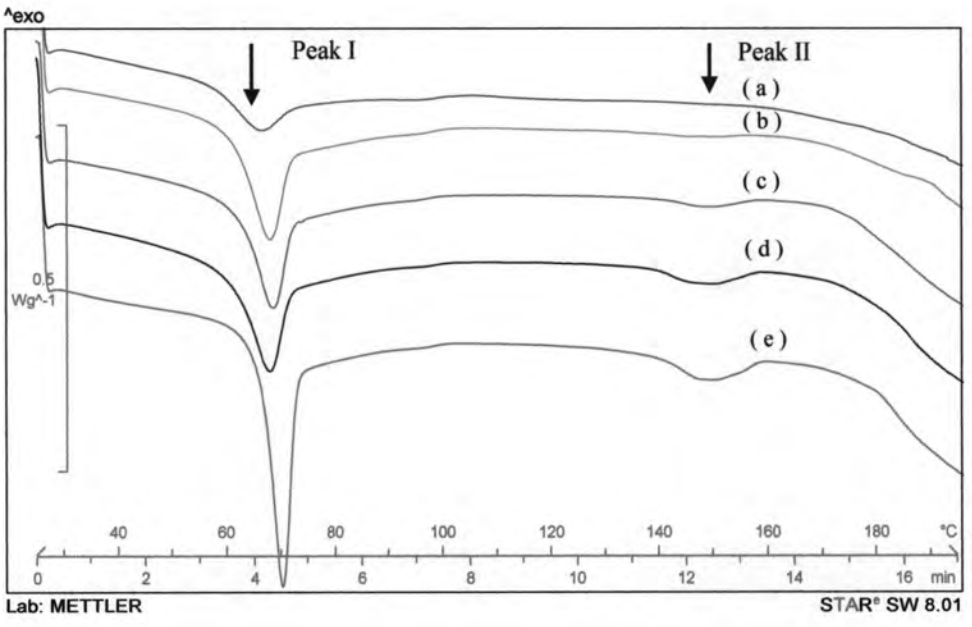

Figure 23 DSC thermograms of amorphous glibenclamide tablets after storage at 60°C ; amorphous glibenclamide after melt quenching (a),1 weeks (b), 2 weeks (c), 3 weeks(d) and 4 weeks(e).

Table 3 Endothermic energy (ΔH) of amorphous glibenclamide powder and tablets after storage at temperature 50 °C as a function of time.

Sample	Storage time	Melt Endothermic			
		Peak I(°C)	ΔH (J/g)	Peak II(°C)	ΔH (J/g)
Amorphous glibenclamide Powder	1 week	68.93	4.81	-	-
	2 week	69.76	5.58	-	-
	3 week	69.59	5.91	-	-
	4 week	70.89	8.11	-	-
Amorphous glibenclamide Tablets	1 week	63.59	3.25	-	-
	2 week	64.09	3.96	-	-
	3 week	64.09	4.11	-	-
	4 week	69.56	5.16	-	-

Table 4 Endothermic area (ΔH) of amorphous glibenclamide powder and tablets after storage at temperature 60 °C as a function of time.

Sample	Storage time	Melt Endothermic			
		Peak I(°C)	ΔH (J/g)	Peak II(°C)	ΔH (J/g)
Amorphous glibenclamide Powder	1 week	67.12	4.09	142.45	-
	2 week	67.10	4.20	145.42	0.62
	3 week	65.76	4.88	145.72	1.11
	4 week	68.57	6.81	149.54	2.38
Amorphous glibenclamide Tablets	1 week	67.74	6.41	-	-
	2 week	68.26	6.03	148.10	0.47
	3 week	67.74	6.13	149.07	1.33
	4 week	70.04	8.38	147.91	2.37

1.4.3 Visual Observation

The amorphous glibenclamide tablets were kept at 50°C and 60°C. The samples were collected every week and monitored by visual observation.

Photograph in Figure 24 shows the initial of amorphous glibenclamide tablet and Figure 25 shows the change in physical transformation to glass-like character of partial crystalline phase. Amorphous glibenclamide tablets stored at 50°C show opaque white color similar to the initial tablet, whereas at 60°C the translucent glass-like characteristics are observed.

Figure 24 The initial amorphous glibenclamide tablets.

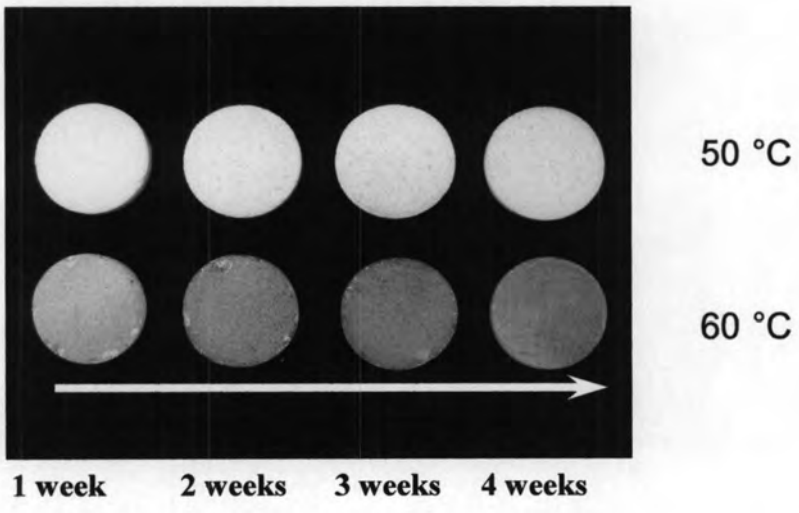

Figure 25 Physical transformation of glibenclamide amorphous tablets after storage at 50°C (top) and 60°C (bottom) during different time periods.

1.4.4 Equilibrium solubility of glibenclamide

In this study the solubility profile of amorphous glibenclamide was investigated by shaking samples of 100 mg amorphous glibenclamide powder in 200 ml phosphate buffer pH 7.4 in sealed Erlenmeyer flasks at 37°C±0.5°C. The samples are withdrawn, the solid is filtered and the liquid assayed at times 1, 3, 6, 9, 18, 30, 42, 54, 72 and 96 hours in triplicate. Figure 26 and Table 16 (Appendix A) show the concentration of glibenclamide which were not increased after 30 hours and defined as the equilibrium solubility of amorphous glibenclamide at 0.240 mg/ml.

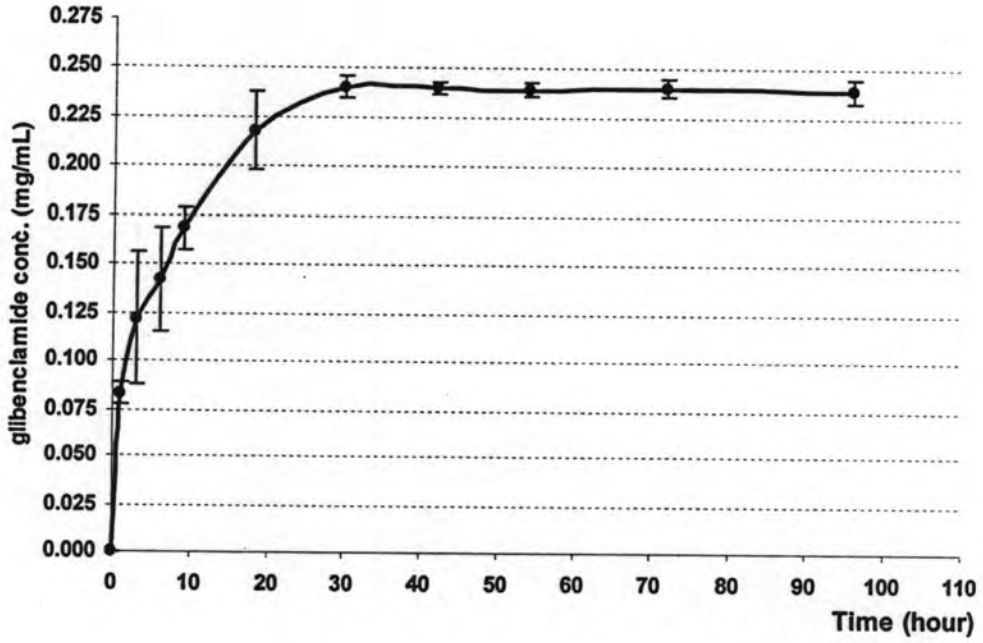

Figure 26 The solubility profile of amorphous glibenclamide powder in phosphate buffer pH7.4 at 37°C±0.5°C

This solubility profile was selected as reference pattern to compare the equilibrium solubility of amorphous glibenclamide after storage at 48 hours at different temperatures.

The equilibrium solubility of crystalline glibenclamide in phosphate buffer (pH 7.4) at 37±0.5°C after 48 hour was 0.013 mg/ml. The amorphous form shows a solubility of 0.267 mg/ml, indicating a 20-fold increase in solubility.

This experiment was to study the effects of storage time and temperature on amorphous glibenclamide powder and tablets. The samples were kept at 50°C and 60°C for 4 weeks. Over a period of every one week, the samples were collected for equilibrium solubility determination.

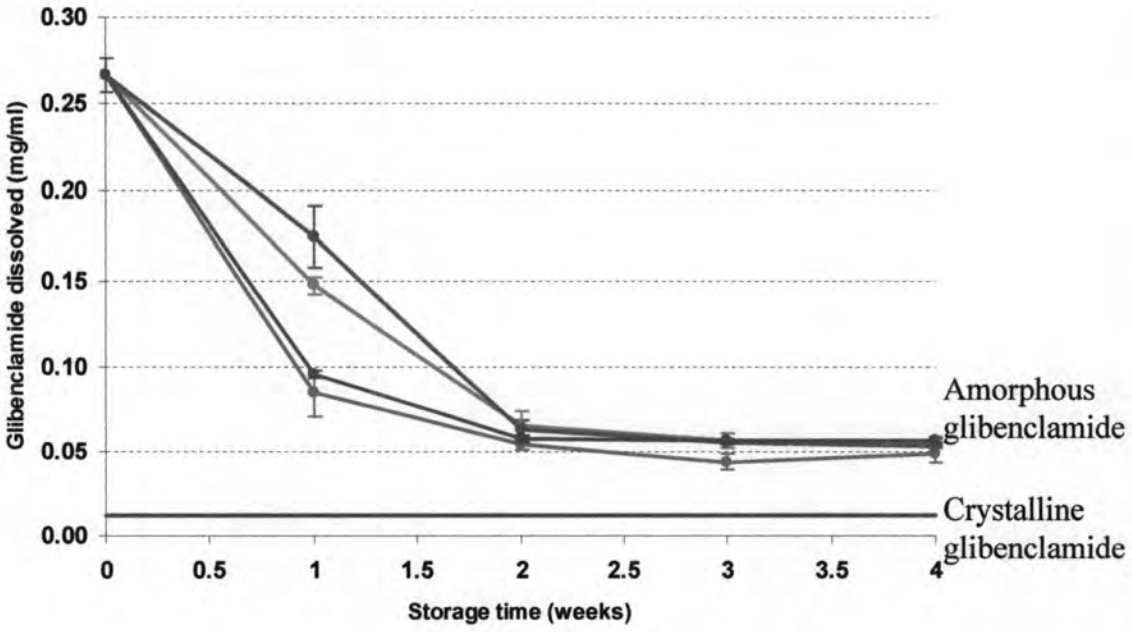

Figure 27 Equilibrium solubility of glibenclamide amorphous after storage at different temperatures as a function of time: amorphous glibenclamide powder at 50°C(●), amorphous glibenclamide tablet at 50°C(●), amorphous glibenclamide powder at 60°C (●), amorphous glibenclamide tablet at 60 °C (●) and crystalline glibenclamide (▬)

The equilibrium solubility of amorphous glibenclamide stored at different temperature as function of time show a curvilinear relationship in Figure

27 which indicates that, for any storage temperature, the solubility initially decreases as the storage time increases up to 2 weeks. However, as the storage temperature increases from 50 to 60 °C the extent of decrease in solubility increases. Increasing the storage time for more than 2 weeks the results shows very little reduction in solubility.

The reduction in solubility of glibenclamide amorphous when stored at different temperatures was attributed to the partial transformation of glibenclamide from the amorphous state to the crystalline state. Amorphous state is more energetic and less stable than the crystalline state as indicated by the heat of fusion ΔH_f values. The ΔH_f as calculated from the endothermic peak of DSC data were 3.26 J/g and 117.73 J/g for amorphous and crystalline states, respectively (Figures 16 and 14).

1.4.5 Chemical degradation of amorphous glibenclamide.

According to the HPLC analysis of glibenclamide received resulted in the retention time 9.3 minutes and was not able to detect other peaks of impurities or related substances.

In order to study the effect of heat during melting of glibenclamide whether it produced related substance or not, HPLC analysis was used. The HPLC chromatogram was shown in Figure 40 (Appendix A). The quench cooled sample shows one retention time of 3.8 minutes, which calculated as 22.18% of chemical degradation had occurred. It has been reported previously by Patterson et al. (2005) that 16.04($\pm$1.55) % chemical degradation in the sample after melting and quench cooling process. As a result of the significant chemical degradation, the quench melts samples show slight color changed in optical property of the solid forms.

The surface morphology of amorphous glibenclamide tablets shows the change in the optical property of solid dosage form which did not resulted

from the increasing of impurities. Because the related substances can be detected by using HPLC analysis and the unknown impurity peak is identified after post process melting and quench cooling. It was due to the fact that the amounts of impurity of sample which was kept at condition 50°C and 60°C and the initial tablets were very similar with only small differences as presented in Table 5 and Figure 28.

Table 5 Percent degradation of amorphous glibenclamide powder and tablets stored at temperatures 50°C and 60°C as a function of time (Appendix A).

	Percent degradation of amorphous glibenclamide				
Condition	Week 0	Week 1	Week 2	Week 3	Week 4
Powder / at 50°C	22.18	24.09	24.31	21.80	24.43
Powder / at 60°C	22.18	22.83	23.72	26.19	26.57
Tablet / at 50°C	22.18	23.22	20.73	25.37	25.72
Tablet / at 60°C	22.18	25.31	26.99	24.45	25.90

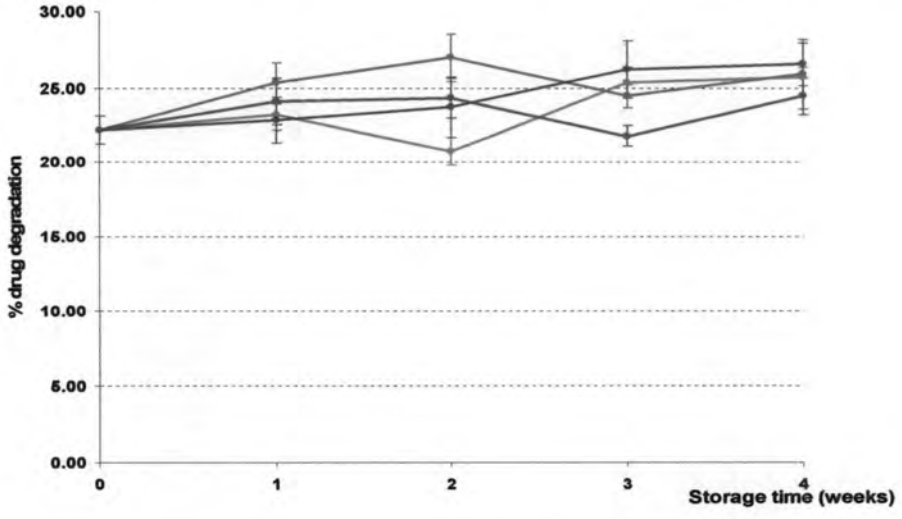

Figure 28 Percent degradation of amorphous glibenclamide powder and tablet stored at temperatures 50°C and 60°C as a function of time. amorphous glibenclamide powder at 50°C(●), amorphous glibenclamide tablet at 50°C(●), amorphous glibenclamide powder at 60°C (●), amorphous glibenclamide tablet at 60 °C (●)

In summary, the endothermic peak at 150°C of DSC thermograms and the reduction of equilibrium solubility were used as a criteria for detect amorphous-crystalline transformation of glibenclamide. The amorphous glibenclamide tablets were prepared by direct-compression of amorphous glibenclamide powder to eliminate the effect of excipient stabilization.

2. Acetylsalicylic acid (ASA, Aspirin) and salicylic acid (SA)

2.1 Analysis of aspirin and salicylic acid by high performance liquid chromatography (HPLC)

Analytical 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 linearity, precision and specificity. The results were shown in Appendix B.

Linearity

The regression coefficient (R^2) for standard curve of aspirin was 0.9997 and salicylic acid was 0.9999. This resulted showed a good linearity of peak area and standard concentration.

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. Precision of the method was expressed as the percentage of relative standard deviation (%RSD) and the data were shown in Tables 24 and 26(Appendix B). The %RSD of the aspirin solution on 3 days was in the range

of 0.190 to 0.425. The %RSD of the salicylic solution on 3 days was in the range of 0.209 to 0.372. The low %RSD indicated the good precision of this method.

Specificity

The resolution between aspirin and salicylic acid peak was 5.6. Tailing factor of aspirin peak was 1.016 and salicylic acid was 1.447. The resolution (>1.5) and tailing factor (<2.0) indicated the good system suitability of this method. The data are shown in Tables 27(Appendix B).

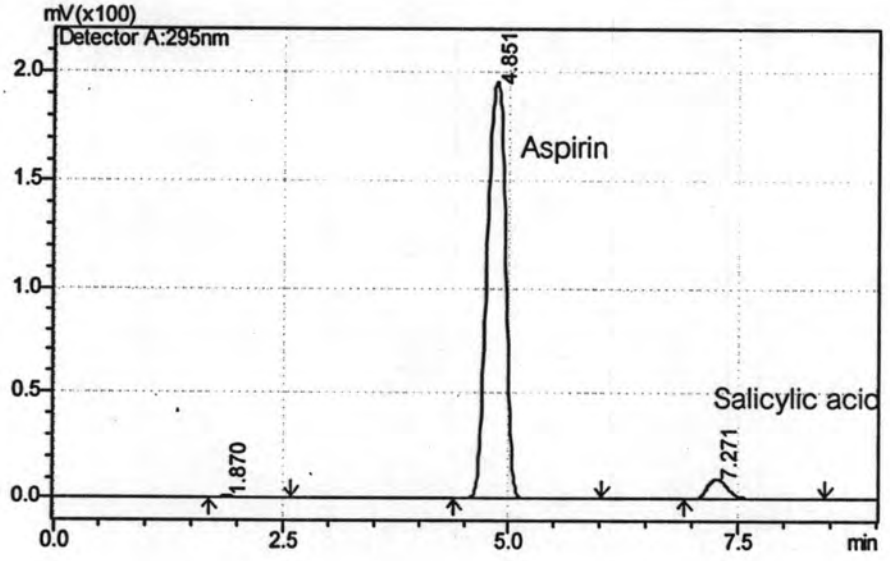

Figure 29 HPLC chromatogram of aspirin and salicylic acid

Determination of reference aspirin (ASA) and salicylic acid (SA) found the retention times in the chromatograms were approximately 4.8 minutes for ASA and 7.3 minutes for SA (Figure 29).

Using the equation from linearity standard curve was shown in Figure 42(Appendix B), the concentration of salicylic acid (SA) for each area under the curve

of HPLC was determined. The acetylsalicylic acid degradation product (SA) was determined for each sampling time and temperature.

2.2 Stability of aspirin

The effect of temperature on aspirin degradation was studied at 30°C, 37°C, 45°C, 50°C and 60°C and the storage humidity of 75% relative humidity. The samples were placed into saturated sodium chloride chamber and immediately close the lid then kept in hot oven at a controlled temperature for 8 week and samples were collected every 2 weeks for evaluation.

The kinetics of aspirin degradation was studied by using HPLC for determination of chemical analysis. Though analysis of kinetic data, determine the experimental rate constants and order of chemical reaction were determined.

Therefore, each milligram of salicylic acid present represents the degradation of 1.304 milligrams of aspirin. Since the amount of aspirin initially present is 85.4168 mg per 100 mg of sample and since the amount of aspirin, which has degraded, can be determined, the amount of aspirin remaining can be calculated referring to the equation 3 -6 in from method along part and the data are shown in Table 28 to 32 (Appendix B).

According to The United States Pharmacopoeia (2005) limit of free salicylic acid in aspirin tablet formulation should not be more than 0.3% w/w. Table 6 to Table 10 show the quantity of salicylic acid which were converted to the quantity of aspirin degraded, the data were normalized for 100 milligrams of sample. In this study found that quantity of free salicylic acid was over the limitation at temperature 50°C on week 8 and at 60°C on week 4 of the test as presented in Tables 9 and 10 with asterisk. At other temperatures the free salicylic acid were not higher than limitation during the test period.

Table 6 Experiment data and calculated values for aspirin degradation at temperature 30 °C / 75%RH

Time (weeks)	salicylic acid (% w/w)	Aspirin degraded (%w/w)	Aspirin remaining (%w/w)
0	0.040	0.0522	85.3646
2	0.038	0.0490	85.3678
4	0.039	0.0513	85.3655
6	0.040	0.0516	85.3652
8	0.040	0.0516	85.3652

Table 7 Experiment data and calculated values for aspirin degradation at temperature 37 °C / 75%RH

Time (weeks)	salicylic acid (% w/w)	Aspirin degraded (%w/w)	Aspirin remaining (%w/w)
0	0.040	0.0519	85.3649
2	0.036	0.0464	85.3704
4	0.042	0.0544	85.3624
6	0.049	0.0644	85.3524
8	0.052	0.0683	85.3485

Table 8 Experiment data and calculated values for aspirin degradation at temperature 45°C / 75%RH

Time (weeks)	salicylic acid (% w/w)	Aspirin degraded (%w/w)	Aspirin remaining (%w/w)
0	0.040	0.0519	85.3649
2	0.044	0.0576	85.3592
4	0.055	0.0722	85.3446
6	0.090	0.1168	85.3000
8	0.161	0.2106	85.2062

Table 9 Experiment data and calculated values for aspirin degradation at temperature 50°C / 75%RH

Time (weeks)	salicylic acid (% w/w)	Aspirin degraded (%w/w)	Aspirin remaining (%w/w)
0	0.040	0.0519	85.3649
2	0.061	0.0797	85.3371
4	0.108	0.1405	85.2763
6	0.226	0.2944	85.1224
8	*0.440	0.5735	84.8433

* SA exceeding the pharmacopoeia USP28 limit of 0.3%w/w.

Table 10 Experiment data and calculated values for aspirin degradation at temperature 60°C / 75%RH

Time (weeks)	salicylic acid (% w/w)	Aspirin degraded (%w/w)	Aspirin remaining (%w/w)
0	0.040	0.0519	85.3649
2	0.225	0.2937	85.1231
4	*0.688	0.8973	84.5195
6	*1.486	1.9378	83.4790
8	*2.583	3.3694	82.0474

* SA exceeding the pharmacopoeia USP28 limit of 0.3%w/w.

The kinetics of aspirin degradation as in Tables 6 to 10 can be evaluated by referring to a pseudo-first order reaction:

$$\ln([A_{rem}] / [A_0]) = - k_{app} * t \quad \text{---------- referring to Eq.7}$$

Where; $[A_{rem}]$ is the mass of A remaining and $[A_0]$ is the initial concentration of aspirin. The graphs were plotted between $\ln([A_{rem}]/[A_0])$ as a function of time.

The previously reported, Li, Zhan and Tao (2008) and El-Banna, Daabis and Abd El-fattah (2006) were investigated that the degradation of aspirin was follow the first order kinetics. In Figure 30 quantitative observations of the first order degradation plots for various temperatures indicate that a higher K_{app} (larger slope) results when the temperature increases.

However, in our experiment, the time measurement did not correspond to the exact start of the reaction, because additional time is required for the sample to reach the desired reaction temperature and equilibrium. Therefore, (0,0) was not an actual data starting point and was not used in the regression calculations.

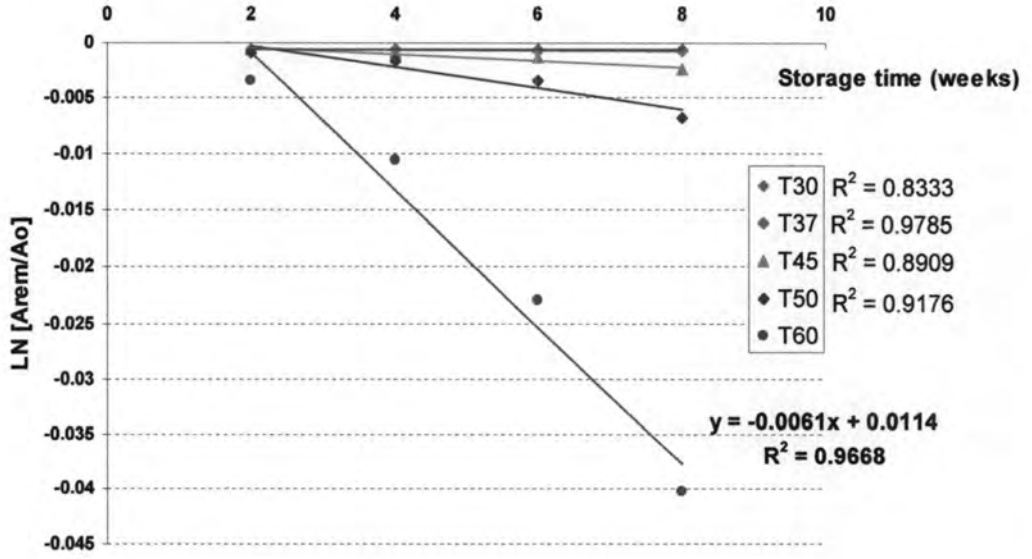

Figure 30 The natural log of $[A_{rem}]/[A_0]$ to time and K_{app} was found as the slope.

As results, temperature 60°C was used to study the developed thin films of degradation indicator. The salicylic acid at 4 weeks was 0.69% which was higher than the pharmacopoeial limit. We used the equation from degradation kinetics at 60°C to estimate the time of the appearance of salicylic acid higher than 0.3% as follows;

$$Y = -0.0061X - 0.0114 \quad \text{----------Eq. 9}$$

The result is 2.6 weeks which is equivalent to 18 days. The time to induce degradation of aspirin to salicylic acid higher than 0.3% at 60°C/75%RH was 18 days.

From two previous experiments, the conditions which were used to induce solid state transformation of glibenclamide was at temperature 60°C for 14 days. Aspirin was degraded when kept at temperature 60°C/75%RH for 18 days. It might be assumed that temperature of 60°C was a dominant degradation condition for both model drugs. Consequently, the future experiments were to develop thin film intended for drug degradation indicator using a temperature sensitive polymer.

3. Encapsulation of Poly-N-isopropylacrylamide and red cabbage color with calcium alginate.

Two techniques were performed to produce thin film as drug degradation indicator.

The first experiment aimed to develop thermally controlled device to detect drug degradation by encapsulation technique. Alginate was selected as the encapsulation material. In general, the solid encapsulated system is produced by initiating droplets of sodium alginate solution into a solution containing divalent metal ions such as $CaCl_2$ to form alginate beads system.

This study was intended to produce matrix system which contained PNIAAm and red cabbage extract in the matrix of alginate beads. PNIAAm was used as a temperature sensitive polymer while, red cabbage extract was a representative of water soluble indicator. Then, the alginate beads were dispersed in HPMC solution to prepare the thin films. However, the red cabbage extract had low entrapment efficiency in the beads due to its water soluble property. The incorporation of the water-soluble substances in beads was not significant because of the surrounding aqueous environment, which caused low encapsulation and rapid release due to the pore size and inherent solubility of the substance in the medium. Therefore, the red cabbage extract had to be prepared in an insoluble form before entrapment in the alginate-PNIAAm beads.

There are two methods which were used to produce the red cabbage in a water insoluble form.

3.1 Encapsulation of red cabbage extract with ethylcellulose polymer by precipitation method

Preparation of the red cabbage extract in water insoluble microspheres was performed by precipitation technique. Ethylcellulose was chosen as an insoluble

polymer to encapsulate the red cabbage extract in the microspheres. These microspheres were harvested by precipitation technique and then encapsulated in the alginate PNIAAm system. The alginate beads which contained PNIAAm and the red cabbage extract microspheres were then harvested by filtration.

3.1.1 Evaluation of temperature sensitive indicator microspheres prepared by precipitation.

The color change of the red cabbage extract in the beads was evaluated by physical observation. The beads were added in 0.1 normal potassium bicarbonate and kept at two temperatures which were at ambient temperature and 80°C for 24 hours. The result revealed that the release rates of the encapsulated beads in both conditions were not different.

It could be concluded that the red cabbage extract was released from the encapsulated microspheres by diffusion controlled mechanism and the temperature did not affect the release rate. Therefore, the red cabbage extract had to be prepared in water insoluble form by other possible methods.

3.2 Encapsulation of red cabbage extract by emulsification method

The red cabbage extract was prepared in the form of water in oil emulsion before used in the encapsulated beads. The red cabbage extract were dissolved in the water phase while, isopropyl myristate served as the oil phase. Span 80 a low HLB surfactant was used in the range of 0.1%-40% w/v solution as emulsifier. The primary emulsion was subjected to stirring at 1,000 rpm for 60 seconds until dispersed homogeneously. It was found that 5%w/v span 80 solutions gave stable primary emulsion system. The span 80 concentration reached 20%w/v the primary emulsion was too viscous to be effectively dispersed in aqueous phase.

The next preparation method was to disperse the emulsion in the aqueous external phase which contained sodium alginate and PNIAAm. Then, the

final multiple emulsion was sprayed into calcium chloride solution to generate calcium alginates/PNIAAm solid complex wall formation. The size of the calcium alginates/PNIAAm complex beads was in the range of 100-500 micrometers.

3.2.1 The evaluation of temperature sensitive indicator microspheres prepared by emulsification method.

The color change of the red cabbage extract was evaluated by physical observation. Addition of the solid complex in 0.1 normal potassium bicarbonate was done and divided into 2 groups to be kept at ambient temperature and 80°C for 24 hours. The color change of the solid complex which was kept at 80°C turned from purple to green while, the color change of the solid complex at ambient condition was not different from the initial. However, the color changes were not clear by physical observation due to the turbidity of the emulsion. Hence, the use of emulsion method to prepare water insoluble red cabbage form before encapsulating in the alginate beads was not practical. It was not only the unclear detection of the release rate from the beads, but also uniformly reproduced. In addition, the percentage of encapsulation could not be evaluated.

In summary, the alginate technique was not suitable for the encapsulation of PNIAAm and the red cabbage extract to develop thermally controlled device. Consequently, the future experiment was to develop thin films by simple layering technique as the thermally controlled as drug degradation indicator.

4. Thin films by layering technique as drug degradation indicator

The proposed design is based on the idea that the film matrix exposed to temperature may lead to the diffusion of red cabbage extract through contracted PNIAAm barrier and interact with the alkaline bottom layer. The PNIAAm served as a valve regulating the permeation of red cabbage extract in response to temperature change.

The approach was to prepare thin films in three layers by using hydroxypropyl methylcellulose as a film base. The first layer contained red cabbage extract in HPMC, the middle layer contained PNIAAm at different concentrations in HPMC acting as polymeric barrier and the last were alkaline in HPMC layer (Figure 31).

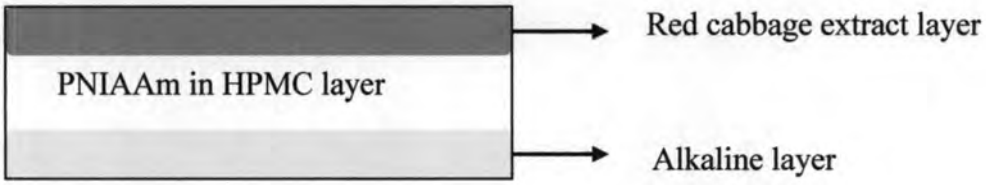

Figure 31 Different layers of thin film produced; red cabbage extract film (top), PNIAAm film (middle) and alkaline film (bottom).

4.1 Preparation of thin films by layering technique.

The films were prepared by a layering method. The films were cast by weighing 20 grams of appropriate composition and concentration of HPMC polymer solution in a glass plate diameter 9.5 cm and each layer films were dried at ambient temperature separately and finally assembled together using HPMC solution as adhesive. Base on the physical property of PNIAAm film, concentration range of 0 to 1 %w/w were chosen as the model polymer to produce microporous structure in the middle layer. The control film sample was prepared using HPMC without PNIAAm. Various formulations of thin film compositions are shown in Table 11. The change in color of thin films was evaluated at 60°C and the relative humidity was approximately 20 %RH as function of time until 24 days period. The experiment was done in triplicate.

Table 11 Concentrations of each layer of the film formulations.

Film Formulation No.	1st Layer Red cabbage extract (% w/w)	2nd Layer PNIAAm (%w/w)	3rd Layer $KHCO_3$ (%w/w)
Control film for F1-F4	0.2	0	0.2
F1	0.2	0.25	0.2
F2	0.2	0.50	0.2
F3	0.2	0.75	0.2
F4	0.2	1.00	0.2
Control film for F5-F8	0.5	0	0.2
F5	0.5	0.25	0.2
F6	0.5	0.50	0.2
F7	0.5	0.75	0.2
F8	0.5	1.00	0.2

4.2 Characterization of thin films as drug degradation indicator

We have prepared thermo-sensitive thin films of red cabbage extract and PNIAAm hydrogel in hydroxypropyl methylcellulose (HPMC) carrier. The phase transition temperature of PNIAAm hydrogel was characterized by differential scanning calorimetry (DSC), and the swelling efficiency was measured at different temperatures.

4.2.1 Thermal analysis of thin film

Thermal behaviors of the PNIAAm and film formulations were evaluated using DSC. The phase transition of thermo-sensitive PNIAAm occurred with the change in the environmental temperature accompanied with a special critical heat of phase transition. The film F3, 0.75% PNIAAm in HPMC film with Polyethylene glycol 400 as plasticizer was used as a representative to detect the change in phase transition temperature.

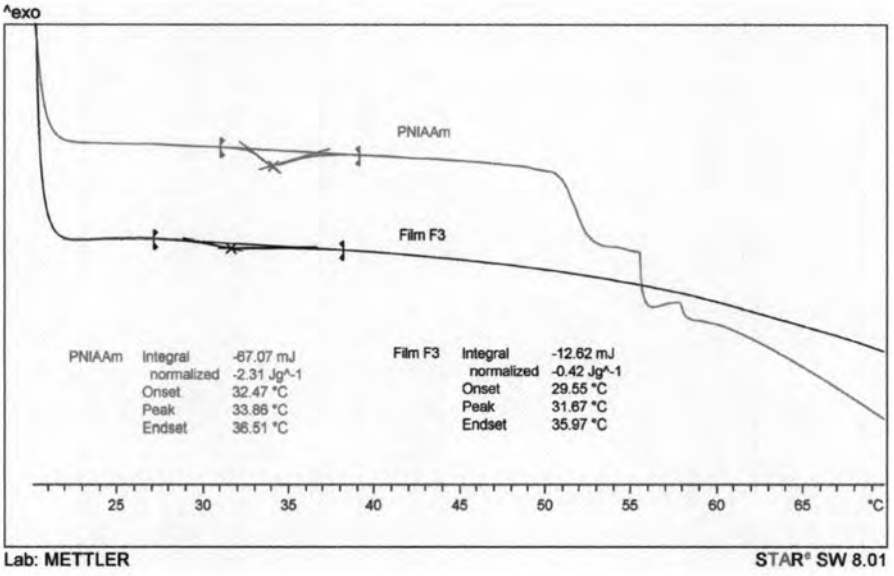

Figure 32 DSC thermograms of PNIAAm and 0.75% w/w PNIAAm in 5% w/w HPMC film (F3).

Figure 32 shows DSC thermograms of PNIAAm, the phase transition is presented at approximately 34°C and revealed only one minor endothermic peak onset at 31°C, corresponding to the phase transition temperature and was defined as lower critical solution temperature (LCST).The phase transition temperature of F3 was slightly lower than PNIAAm polymer. It could be concluded that the effect of plasticizer may have reduced the phase transformation of PNIAAm in this film formulation.

4.2.2 Thickness evaluation

Film thickness is considered as one of the most important parameter to control thin films used in tablet degradation indicator. The thickness of each dried film was monitored. Thin film thickness was measured using a micrometer or vernier caliper. At least six measurements of the thickness were made at different locations. Thickness will be the average of all the measurements. The average thickness of HPMC film containing the red cabbage extract were 0.20±0.02 mm, the alkaline film were 0.20±0.02 mm and the PNIAAm films are shown in Table 12. The concentrations of PNIAAm were varied in 0.25, 0.50, 0.75 and 1.00 %w/w. The PNIAAm film at the concentration of equal or more than 1.25 %w/w showed non uniform distribution of polymer and aggregation of polymer at different locations were observed.

4.2.3 Percent moisture sorption

PNIAAm in HPMC layer was determined for its moisture absorptive property. According to Table12, at temperature 25±2 °C, percent moisture sorption of the HPMC, PNIAAm and all of film formulations were not different (approximately 51-54 %w/w). At 60 °C, PNIAAm polymer absorbed moisture only approximate 13%w/w, while HPMC was 37%w/w. When the PNIAAm content was increased, there were decreased in the film moisture sorption. At the temperature of 60 °C PNIAAm seemed to have the minimum moisture sorption. It could be concluded that, higher extent of aggregation or deswelling in this PNIAAm network at 60 °C leading to reduce moisture sorption, resulted from the shrinking in structure of the polymer chains.

Table 12 The average thickness and percent moisture sorption at room temperature and at 60°C of PNIAAm in hydroxypropyl methylcellulose film.

Content of PNIAAm in HPMC dry film (2cm. x 2cm.)	Average weight (mg) ± SD (n=6)	Thickness of film average (mm) ± SD (n=6)	(%)Moisture sorption at 25 °C ± 2 °C			(%)Moisture sorption at 60°C		
			1	2	Ave.	1	2	Ave.
1. HPMC film (control)	93.0 ± 4.3	0.20±0.02	55.25	54.18	54.71	38.28	37.34	37.81
2. HPMC 70.52 mg PNIAAm 3.5 mg (Formulation F1)	96.5 ± 4.3	0.21±0.02	52.33	53.90	53.12	30.71	32.80	31.75
3. HPMC 70.52 mg PNIAAm 7.0 mg (Formulation F2)	97.9 ± 5.6	0.27±0.03	54.78	49.01	51.89	29.41	25.79	27.60
4. HPMC 70.52 mg PNIAAm 10.5 mg (Formulation F3)	100.1 ± 7.4	0.38±0.04	54.78	53.09	53.93	22.14	20.00	21.07
5. HPMC 70.52 mg PNIAAm 14.5 mg (Formulation F4)	105.6 ± 8.8	0.47± 0.03	53.28	56.45	54.87	19.63	20.41	20.02
6. HPMC 70.52 mg PNIAAm 17.5mg	98.0 ± 9.0	0.51± 0.07	52.72	56.30	54.51	21.92	24.39	23.16
7. PNIAAm Powder (control)	50.6 ± 1.8	-	51.00	53.48	52.24	13.58	12.30	12.94

4.2.4 The thin films color evaluation

The films were prepared by layering method and each films were dried at ambient temperature separately, cut into rectangles (1cm x 0.5cm) and finally assembled together using HPMC solution adhesive.

In this experiment, 60°C was used as the temperature to determine the indicator efficiency because at this temperature the red cabbage color was expected to diffuse through PNIAAm film layer and reacted with alkaline layer. This temperature correlated well with the serious equilibrium solubility change of glibenclamide due to solid state transformation and also correlated with the length of time needed to reach the pharmacopoeial chemical degradation limit of aspirin. The thin films were placed in hot air oven at a constant temperature of 60°C and the relative humidity of approximately 20 %RH for 24 days and collected every 3 days for evaluation.

The thin films were placed into a Lexmark X83 scanner and processed using the Photoshop® software. Photoshop® was use to separate each of the thin films sample into ten positions as shown in Figure 33. For each position, the a* value was obtained from the Info Palette function. Since a* value represents the redness, the degradation of red color during storage of all systems and the reaction of red color with alkaline of all film formulation were verified by the decrease in a* values.

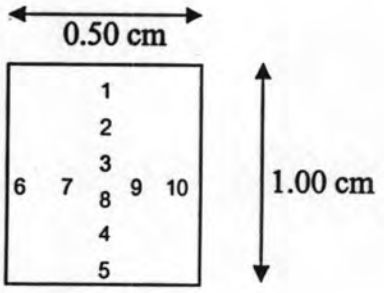

Figure 33 The positions observed within the sample indicator films.

All thin film formulations were kept in a dry place with silica gel and protected from light at ambient temperature (25°C) for 2 months. The a* value and visual observation were evaluated and the data are shown in Table 33 and 34 (Appendix C). The a* value showed small difference between the initial and the final value. The visual observation of the thin films was similar to initial. The rate of color change of thin films were very slow at 25°C, it might be because the PNIAAm remained swollen in the internal cavities which obstructs the release of red cabbage extract through the PNIAAm barrier membrane. At this temperature, the polymer films were not dehydrated, the space between red cabbage film and alkaline film were more than storage at 60°C.

The change in color of thin film as indicators at 60°C were evaluated as function of duration of exposure at 3, 6, 9, 12, 15, 18, 21 and 24 days and the experiment was done in triplicate.

The plot between a*value and time were used to compare the difference between each film formulations. The end point were evaluated using the a*value from thin films of red cabbage layer directly attached on the alkaline layer and stored at temperature of 60 °C and humidity of approximately 20 %RH for 24 days. As a result, film formulations F1-F4 and formulations F5-F8 presented a*values with of the final end point of -1.7 and -3.1, respectively.

The initial a* value of film formulations with the first layer prepared from red cabbage at a 0.2 %w/w and 0.5%w/w were slightly different because the color of the higher concentration of red cabbage film showed only more intense hue of the same color tone. The a* value will not represent the brightness or the lightness of color.

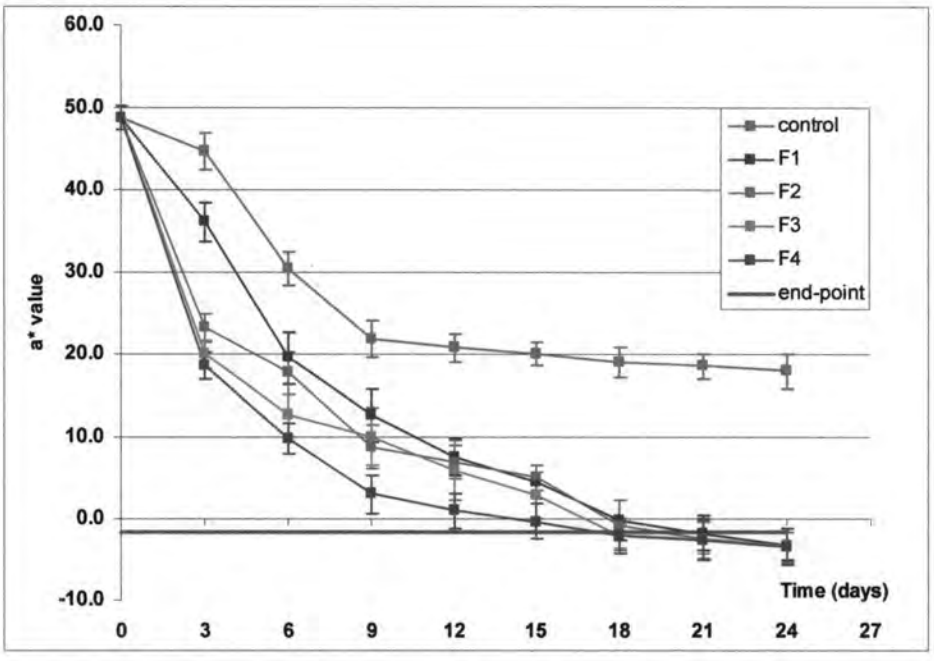

Figure 34 Effect of PNIAAm concentration in thin film on a* value during storage at 60°C as a function of time with the first layer prepared from red cabbage at a 0.2 %w/w fixed concentration.

The plot in Figure 34 shows that film indicators gradually loss the red color parameter a*value as a function of time. The a*value of the film end point was at approximately -1.70. In this Figure, every F1, F2, F3 and F4 formulations (0.2%w/w red cabbage extract) showed a continuous decrease of a* value. For every film formulations, the result indicated that the a* value reached the end point at 18 days. However, the a* value of F4 reached end point earlier at only 15 days.

The area under the curves were calculated by integration of a* value from time 0 to 24 days for each formulations. The mean area under the curve were evaluated by using one-way ANOVA and were found to be significantly different ($p<0.05$), as shown in Table 38 (Appendix C).

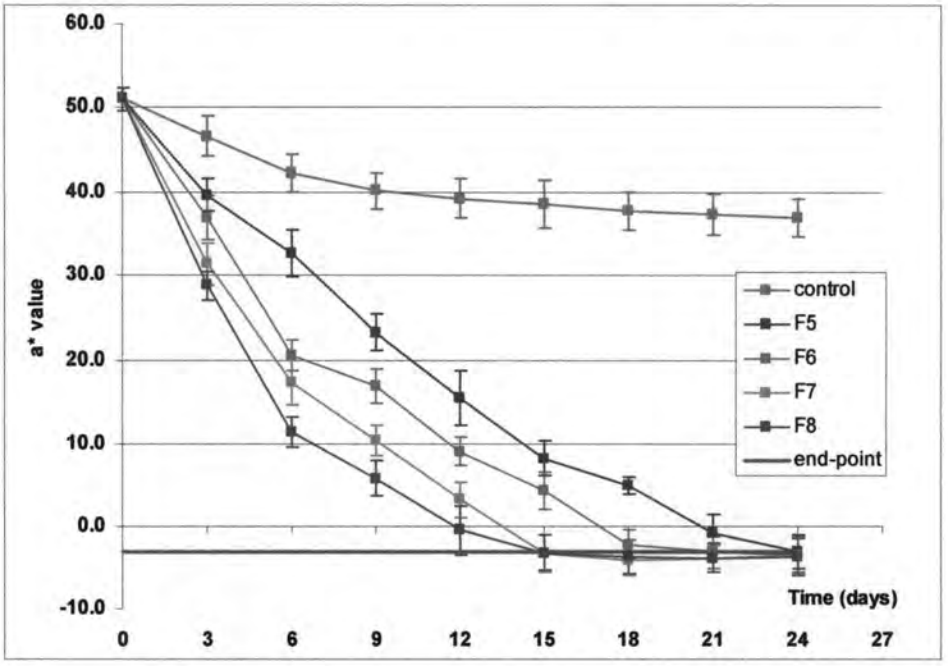

Figure 35 Effect of PNIAAm concentration in thin film on a* value during storage at 60°C as a function of time with the first layer prepared from red cabbage at a 0.5 %w/w fixed concentration.

In Figure 35, for formulations F5, F6, F7 and F8 showed continuous decrease in a* value. As a result, the a* values reached the end point at 15 days for F7 and F8, at 18 days for F6 and at 24 days for F5. The area under the curves were calculated by integration of a* value from time 0 to 24 days for each formulations. The mean area under the curves were evaluated by one-way ANOVA and were found to be significantly different ($p<0.05$), as shown in Table 40 (Appendix C).

The color change in film formulations F5-F8 was slower than films formulation F1-F4 because the color of red cabbage in concentration 0.5 % w/w could retained the red color for longer period than at low concentration. In addition, the red cabbage color in concentration of 0.2 %w/w resulted in the browning of red cabbage color and interfered with the subsequent color evaluation of thin films.

A model independent approach using a difference factor (f_1) and a similarity factor (f_2) was utilized to compare reference color and the color test

profiles. The difference factor was defined in the "FDA Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms" (http://www.fda.gov/cder/guidance.html, 2007) as "calculation of the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves":

$$f_1 = 100 [\text{sum } (Rt-Tt)]/ [\text{sum } Rt] \quad \text{----------Eq.10}$$

The similarity factor is a measurement of the similarity in the percent release between the two curves and is defined as the "logarithmic reciprocal square root transformation of the sum of the squared error" in the FDA Guidance document

$$f_2 = 50 * \log [\{1+ (1/N)*\text{sum } (Rt-Tt)^2\}^{-0.5}*100] \quad \text{----------Eq.11}$$

Where N is the number of time points, Rt is the experimental data at time t, and Tt is the predicted in vivo percent cumulative release at time t.

It is noted in the FDA Guidance document that generally, f_1 value lower than 15 and f_2 value greater than 50 suggest equivalence of the two profiles.

The results of difference factor and similarity factor for formulations F1-F4 showed the difference between each thin film formulations and their control formulation. Films formulations F5-F8 also showed the difference between each thin films formulations and their control formulation. It could be conclude that the a*value profile of each film formulations were different. The results are shown in Table 41 (Appendix C).

The release rate was relatively low at room temperature. However, when the temperature was 60°C, a marked increase in release rate was observed. This trend was found to be reproducible when the temperature was repeatedly altered between room temperature and 60°C. As a result a stepwise response to the temperature alteration was obtained. Below LCST, expanded PNIAAm would close

the pore of the film, resulting in a lower release rate. Above LCST, the thermally contracted polymer would open the pore, resulting in a higher release rate. Therefore, positive thermoresponse controlled release could be obtained in our indicator systems.

The release rate can be mainly driven by the shrinkage of PNIAAm in expanding the space or matching HPMC to be more porous. The difference in the reaction rate of thin film formulations became more noticeable when the content of PNIPAM in the middle layer is higher. Below lower critical solution temperature (LCST), the expanded PNIAAm would close the pores of the films, resulting in as lower release rate of the red cabbage color through the pores. Above LCST, PNIAAm is in a contracted form and the pores were opened causing faster release of the red cabbage extract. In the case of HPMC film, the degree of release through HPMC alone was lower than when PNIAAm was incorporated.

As in the preliminary study the amorphous Glibenclamide equilibrium solubility was significantly different between initial and after storage at 60°C for 2 weeks (14 days). The color of films formulation F7 and F8 change to the end point at the same time at approximately 15 days which correlates well with glibenclamide solid transformation. Thus, F7 and F8 were a good candidate for the future study. The color change profile (a* value) formulation F7 showed higher difference between a* value at 12 days and at the end point of 15 days than formulation F8. Thus, F7 was selected because it was easier to detect the critical end point of F7 by simple visual inspection.

The cutoff point for aspirin tablet was determined by the degradation limit of salicylic acid of over 0.3%w/w. At 60 °C, 75%RH aspirin tablets produce salicylic acid reaching the limit of 0.3% at 18 days. The thin film formulation F6 matched this product because color detection end point of formulation F6 was also at approximately 18 days of test.

The experiment was aimed to design a color marker indicator as drug degradation on the surface of tablets where it will not hinder the release profile of the

tablet. The surfaces of tablets were coated with ethylcellulose to protect the interaction between the drug and excipients and the components in the indicator. The indicator was a thin film which composed of 3 layers. The bottom layer which attached to the tablets was alkaline. The middle layer was PNIAAm barrier and the top layer was the red cabbage extract which placed cross the other layers as shown in Figure 36 (a). The interaction between red cabbage extract and alkaline was represented by the change in color from red to yellow-green, occurred at the intersection as shown in Figure 37 (b) the area of red cabbage film which did not interact with alkaline film was used as a control to compare with interaction was evaluated by the physical observation.

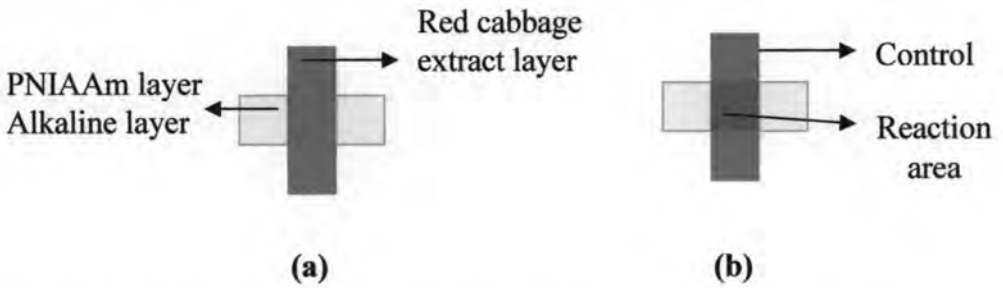

Figure 36 Model of thin films as drug degradation indicator; Initial (a), end point (b)

The thin films as degradation indicator were attached on the surface of tablets. The size of indicator surface area (reaction area) was reduced from 0.5 cm^2 (1 cm x 0.5 cm) to 0.04 cm^2 (0.2 cm x 0.2 cm) for glibenclamide tablets and 0.02 cm^2 (0.15 cm x 0.15 cm) for aspirin tablets. The thin films as drug degradation indicator were attached on the surface of amorphous glibenclamide tablets and commercial aspirin tablets by using HPMC solution as adhesive. The samples were kept at 60°C and observed for color change. The film formulation F7 was used for amorphous glibenclamide tablets. The film formulation F6 was used for aspirin tablets. The color of the indicator reached the end point approximately 12 days. At 7 days both F6 and F7 change color in some part of the indicator. The physical observations showed that the color change of these indicators were not exactly similar to the thin films which have been studies. Because the method for preparing these thin films that were not practically. The HPMC adhesive could interfered and retard with the reaction. Thus,

the small surface area of thin films were affected by the polymer uniformity at intersect point. It was due to the error in the rate of color change in indicator.

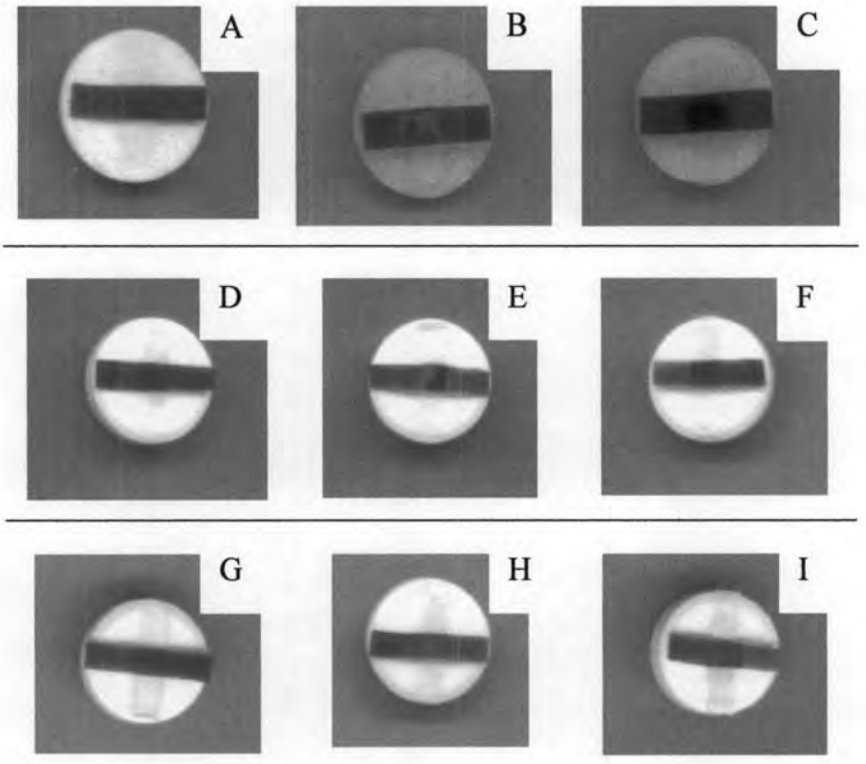

Figure 37 The color of thin films as drug degradation indicator on glibenclamide and aspirin tablets.
Glibenclamide tablets at 60°C/20%RH: Initial (A), at 7 days (B) and at 10 days (C).
Aspirin tablets at 60°C/20%RH: Initial (D), at 7 days (E) and at 12 days (F).
Aspirin tablets at 60 °C/75%RH: Initial (G), at 7 days (H) and at 10 days (I).

The humidity effect on the rate of color change was studied. The aspirin tablets with thin films attach on surface were placed in well close chamber at temperature 60°C/ 75% relative humidity for 20 days. The resulted showed the red color of film had faded and reached the end point at approximately 10 days which was faster than expected. The thin film with fading color was difficult to detect the end point. It might be that HPMC film could absorb moisture at this condition. Thus, the color change rate was faster than when retained at 60°C/20% relative humidity.