CHAPTER IV
RESULTS AND DISCUSSION

1. Preparation of nifedipine spray dried microspheres

From the spray drying conditions used, the nifedipine microspheres spray dried with combined carriers, Eudragit RS100 and Povidone K30 at 55, 65 and 75 ^{o}C, were successfully obtained as yellowish, discrete, dried powder.

The particle size analysis of nifedipine spray dried microspheres was carried out using the image analyzer. Measurement of at least 600 particles for each sample was required for high reliability and accuracy. This method has many advantages over other methods, e.g., it provides a direct measurement, no need of preparing a sample into dispersion. The median diameters in micrometer are shown in Table 2.

Table 2 Median diameters (μm) of nifedipine spray dried microspheres.

Nifedipine : Eudragit RS100 : PVP K30	Median particle size (μm)		
	55 (^{o}C)	65 (^{o}C)	75 (^{o}C)
1:10:0	18.54	18.10	18.17
1:8:2	15.50	14.44	13.87
1:5:5	18.14	13.76	12.88
1:2:8	16.70	14.50	13.13
1:0:10	12.92	12.50	11.31

The microspheres obtained from spray drying had small median diameters ranged from 11.31 - 18.54 μm. It was notable that the microspheres from this method were uniform in shape and size, as the size distribution of all formulas was narrow (Table 2, Tables A1-A28).

From the non-parametric, Kruskal-Wallis test (Table E1), the results shown that there are no significant difference of the median diameter among formulas ($p = 0.450$). However, Table 2 shows roughly that the particle sizes decreased with the ratio of PVP increased. This might be due to the viscosity effect of spray solutions. In spray drying process, it appeared that PVP gave a lower viscosity than Eudragit RS100 at the same concentration. Since Eudragit RS100 has notably higher molecular weight (150,000) than that of PVP K30 (40,000) (Windholz, 1983), the former has higher viscosity. As expected, the higher inlet air temperatures resulted in the smaller microsphere size. This occurred since high temperature usually affect viscosity and surface tension, aiding in the formation of droplets (Swarbrick and Boylan, 1996).

2. Quantitative analysis of nifedipine by HPLC

After several preliminary runs, the most appropriate instrumental conditions were obtained as follows:

Stationary phase	: μ-Bondapak C18 column (3.9 x 300 mm, particle size 10 μm)
Mobile phase	: 6:4 mixture of methanol-water
Flow rate	: 1.2 ml/min
Internal standard	: 4-Dimethylaminobenzaldehyde
Detection wavelength	: 254 nm

Specificity of the HPLC conditions

The chromatogram of the irradiated nifedipine solution in the light cabinet illuminated with daylight fluorescent lamps for 72 h, gave a good resolution between sharp peaks of nifedipine, nifedipine degradation products and the internal standard. The retention times of the internal standard, degradation product, and nifedipine , as shown in Figure 1, were 5.6, 7.3, and 8.7 minutes, respectively. The degradation product detected under these conditions was speculated to be nitrosophenylpyridine compound. As reported by many investigations (Akimoto et al., 1988; Florey, 1989), the degradation products of nifedipine exposed to daylight was nitrosopyridine derivative.

The addition of the compounds under investigation did not interfere the analysis of nifedipine. Figures 2-4 exhibit that there are no any other peaks from tartrazine, sunset yellow, and sodium bisulfite interfering with both nifedipine and internal standard peaks. Curcumin and curcumin crude extract gave no peak at the used concentration.

Calibration curve of nifedipine and linearity of the analysis

The calibration curve of nifedipine was plotted between the average peak area ratio of nifedipine to internal standard versus nifedipine concentration in ng/ml. The calibration curve was prepared individually at each run of HPLC. A typical of calibration curve is shown in Figure 5. The coefficient of determination (R^2) of linear regression line obtained was 0.99997.

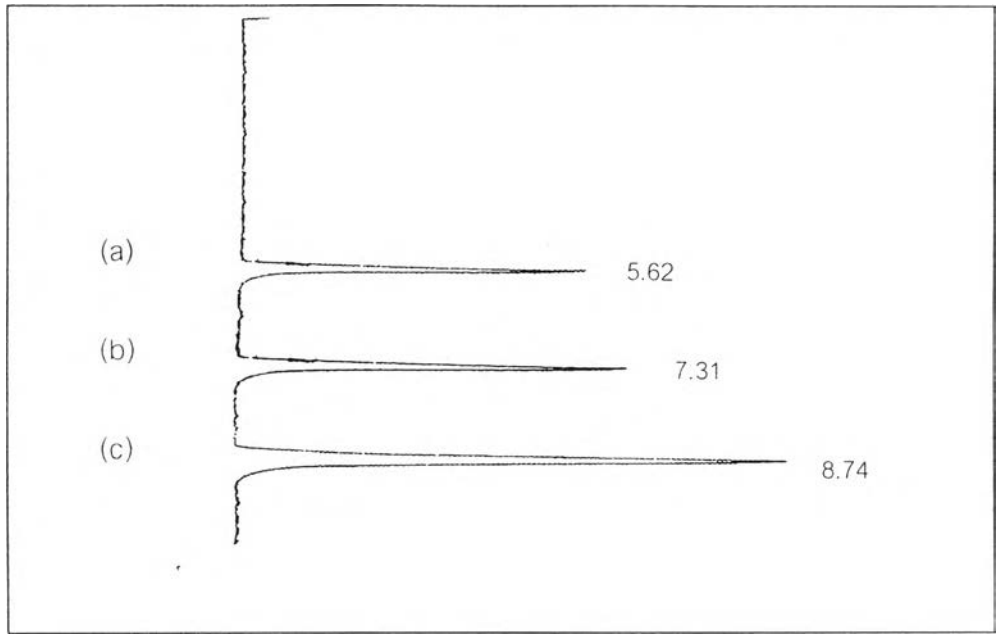

Figure 1 The HPLC chromatogram of internal standard (a), degradation product (b), and nifedipine (c).

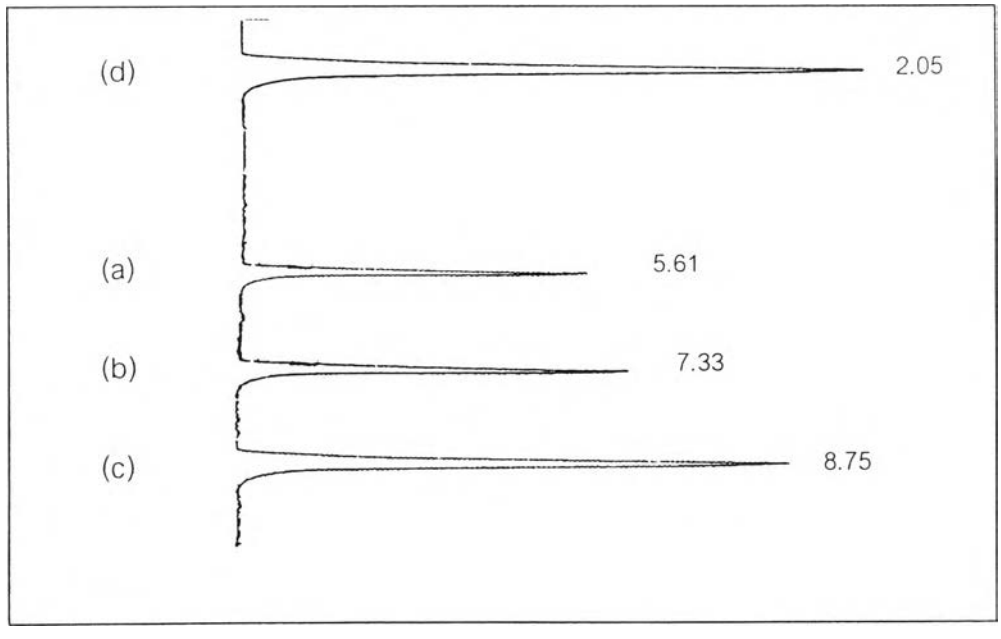

Figure 2 The HPLC chromatogram of internal standard (a), degradation product (b), nifedipine (c) and tartrazine (d).

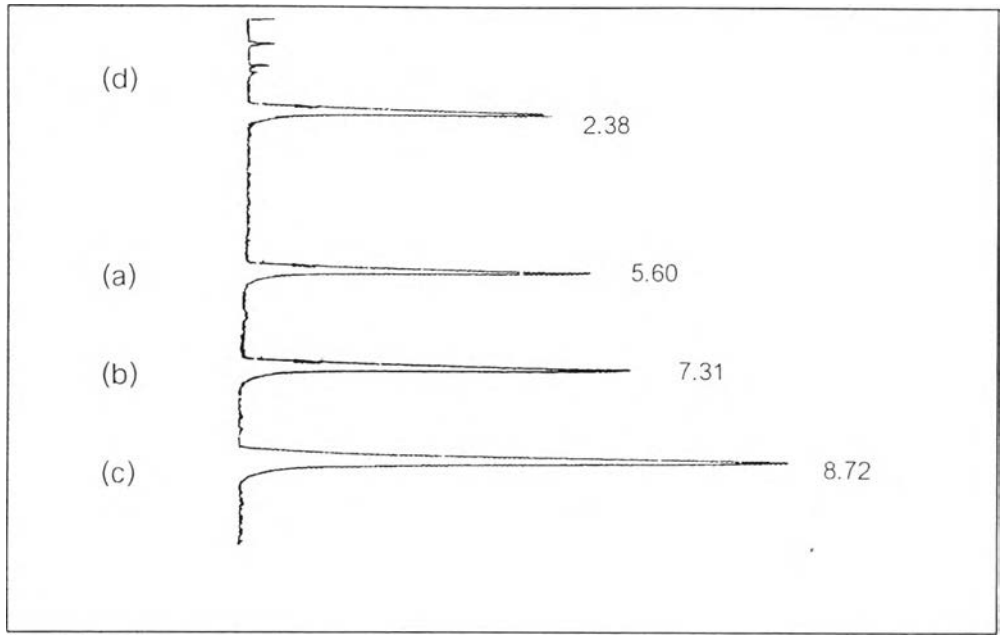

Figure 3 The HPLC chromatogram of internal standard (a), degradation product (b), nifedipine (c) and sunset yellow (d).

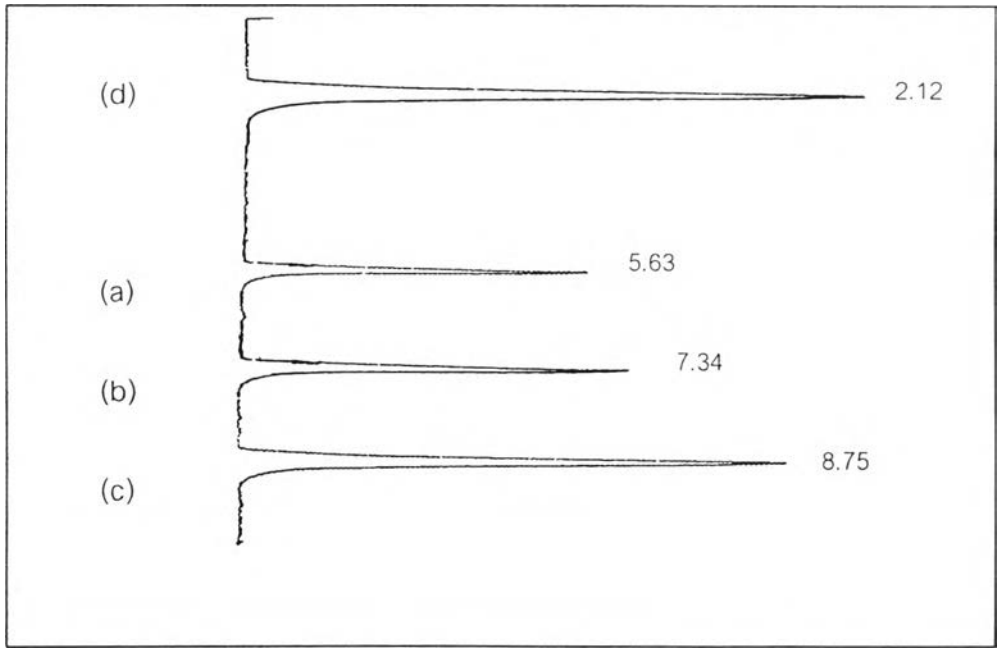

Figure 4 The HPLC chromatogram of internal standard (a), degradation product (b), nifedipine (c) and sodium bisulfite (d).

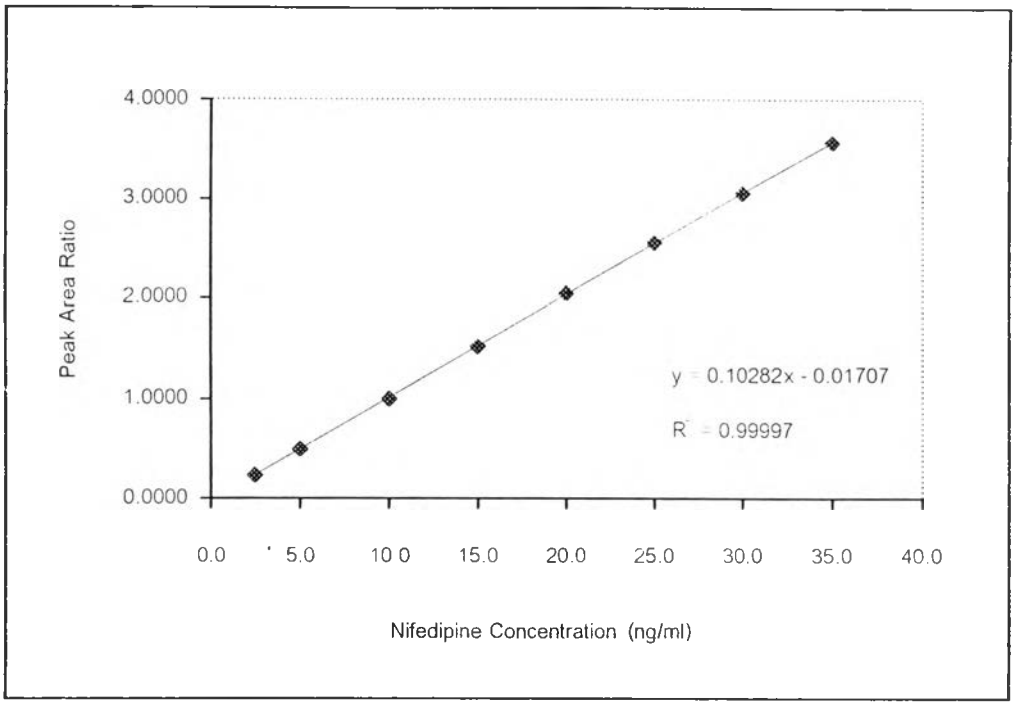

Figure 5 A calibration curve of nifedipine obtained from HPLC method.

Accuracy of the analysis

In order to determine the accuracy of the HPLC method, three series of nifedipine solution were prepared, according to the concentration used in calibration curve determination, and analyzed for nifedipine content by the HPLC method. The differences between the nifedipine concentration calculated from regression line of the calibration curve and the theoretical nifedipine concentration was expressed in percentage analytical (%recovery). The coefficient of variation (%CV) was also calculated from the mean and standard deviation of %recovery. The accuracy of the method was high as shown from the %recovery of 99.07-100.82% (Table 3).

Table 3 Accuracy of the analysis of nifedipine by HPLC method

Nifedipine conc. (ng/ml)	Peak area ratio				Calculated nifedipine conc. (ng/ml) [a]	% Recovery [b]
	No. 1	No. 2	No. 3	Average		
2.5	0.2461	0.2326	0.2416	0.2401	2.50	100.07
5.0	0.5153	0.5040	0.4844	0.5012	5.04	100.82
10.0	0.9954	1.0143	0.9948	1.0015	9.91	99.07
15.0	1.5377	1.5284	1.4973	1.5211	14.96	99.73
20.0	2.0430	2.0475	2.0532	2.0479	20.08	100.42
25.0	2.5562	2.5804	2.5458	2.5608	25.07	100.29
30.0	3.0644	3.0636	3.0679	3.0653	29.98	99.93
35.0	3.5756	3.5746	3.5811	3.5771	34.96	99.87
					Mean	100.02
					S.D.	0.52
					% C.V.	0.52

[a] determined from the slope of graph between nifedipine concentration and peak area ratio.

[b] determined from the calculated nifedipine concentration x 100 / the theoretical nifedipine concentration.

Precision of the analysis

To determine the within-run and between-run precisions of the analysis of nifedipine, three series of nifedipine, with concentration of 2.5, 15.0 and 35.0 ng/ml were prepared and analyzed for nifedipine content. The example data are shown in Tables 4 and 5 for within- and between-run tests, respectively. From the calculated %CV, it was found that the HPLC conditions gave high precision, as shown from the low %CV of 0.65 to 2.69% for within-run precision and 1.12 to 2.39% for between-run precision.

Table 4 Within-run precision of nifedipine analysis by HPLC method

Nifedipine conc. (ng/ml)	Peak area ratio				S.D.	%CV
	No. 1	No. 2	No. 3	Average		
2.5	0.2245	0.2308	0.2187	0.2247	0.01	2.69
15.0	1.5808	1.5233	1.5296	1.5446	0.03	2.04
35.0	3.5651	3.5955	3.5501	3.5702	0.02	0.65

Table 5 Between-run precision of nifedipine analysis by HPLC method

Nifedipine conc. (ng/ml)	Peak area ratio				S.D.	%CV
	No. 1	No. 2	No. 3	Average		
2.5	0.278	0.2734	0.2722	0.2745	0.00	1.12
15	1.5416	1.5675	1.495	1.5347	0.04	2.39
35	3.6109	3.6486	3.5411	3.6002	0.05	1.51

3. Effects of processing, formulation and physical factors on nifedipine photodegradation

3.1 Effects of PVP K30 content and inlet air temperature

After exposure to fluorescent light, the decrement of nifedipine concentration occurred in all formulations. Nifedipine showed high photosensitivity even in the solid state. Nifedipine decomposed very quickly. In most formula, within 20-24 h, more than 90% of the drug degraded. However, further degradation occurred very

slowly during the remaining investigation time (Tables B1-B15). The degradation profile of the 1:2:8 nifedipine:Eudragit RS100:PVP K30 microspheres spray dried at inlet air temperature of 65 ^{o}C was shown in Figure 6 as an example. This observation was found in all formulas. It might be attributable to the sample portion unexposed directly to the light, inspite of the sample thickness of less than 3 mm. To determine the order of reaction, graphs of %nifedipine remaining versus time, ln(%nifedipine remaining) versus time, 1/ (%nifedipine remaining) versus time, and 1/ (%nifedipine remaining)^2 versus time were plotted for zero-order, first-order, second-order, and third-order reactions, respectively. Some examples of the plots of the microspheres were demonstrated in Figures 6-9. Linear regression was used to determine the coefficient of determination (R^2) which were then compared, as shown in Table B16. The results indicated that the plot of ln (%nifedipine remaining) versus time gave the highest coefficient of determination and significant regression coefficients ($p<0.05$). Therefore, the photodegradation of nifedipine spray dried microspheres followed first-order reaction.

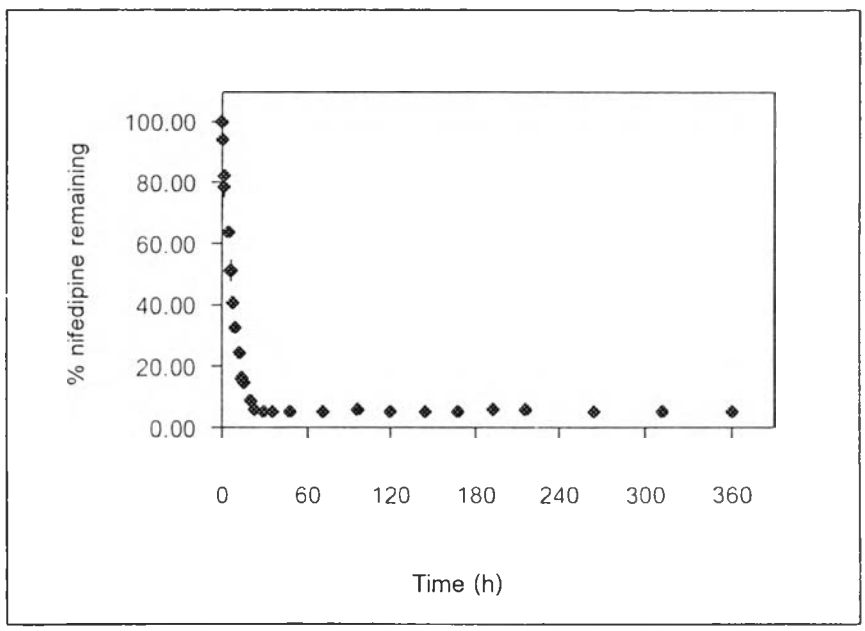

Figure 6 Zero-order plot of photodegradation of 1:2:8 nifedipine:Eudragit RS100:PVP K30 spray dried at inlet air temperature 65 ^{o}C.

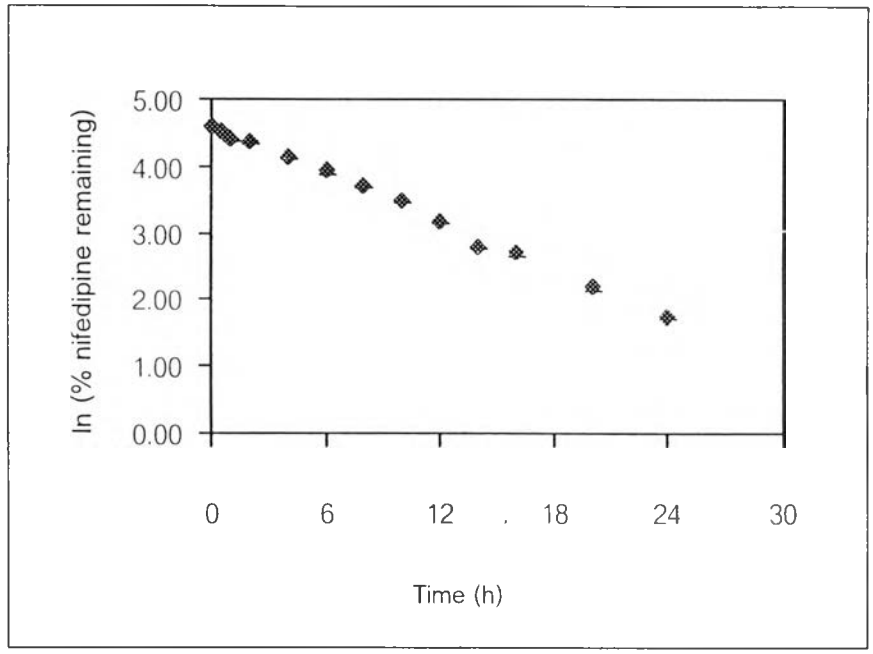

Figure 7 First-order plot of photodegradation of 1:2:8 nifedipine:Eudragit RS100:PVP K30 spray dried at inlet air temperature 65 °C.

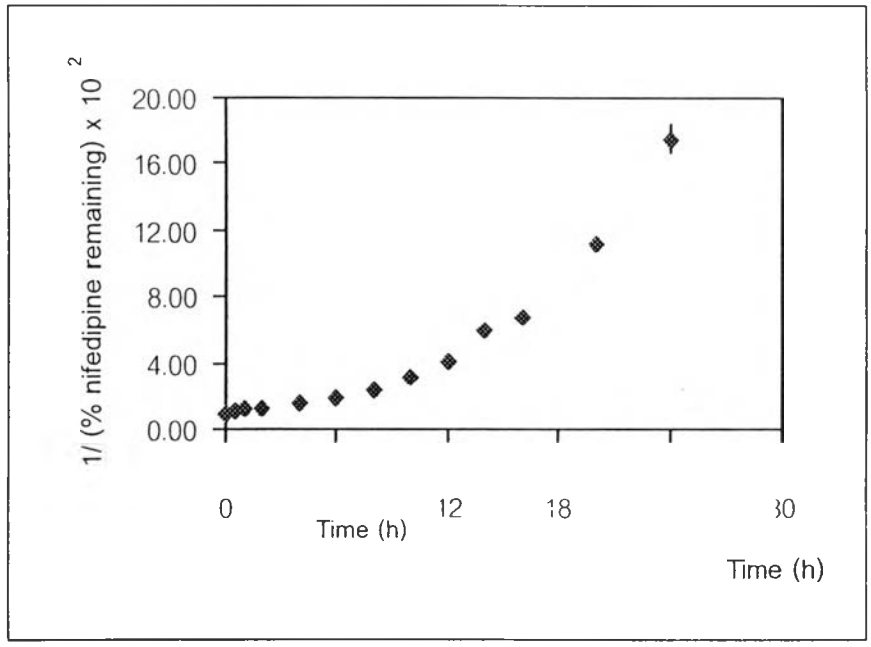

Figure 8 Second-order plot of photodegradation of 1:2:8 nifedipine:Eudragit RS100:PVP K30 spray dried at inlet air temperature 65 °C.

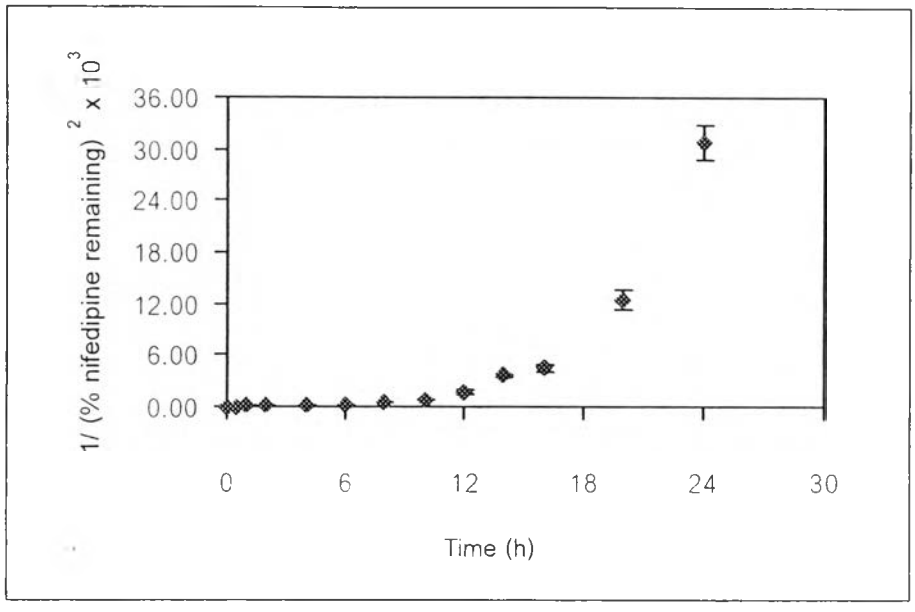

Figure 9 Third-order plot of photodegradation of 1:2:8 nifedipine:Eudragit RS100:PVP K30 spray dried at inlet air temperature 65 ^{o}C.

The degradation rate constants of each formula of nifedipine spray dried microspheres, k (h^{-1}), were calculated from slope of the first-order plots. Their significant differences are tested using three-way ANOVA (SPSS 9.0 for windows) at significant level, α=0.05 (Table E2). It was found that PVP content and inlet air temperature has significantly effect on degradation rate constant of nifedipine microspheres ($p<0.05$). There was an interaction between PVP content and inlet air temperature effect ($p<0.05$), meaning that at the same PVP mixing ratio, degradation constants of microspheres spray dried at varied inlet air temperatures were different. From the multiple comparisons test, it was found that the degradation rate constants of the nifedipine microspheres of 0% PVP K30 content were significant lower than that of other groups at all inlet air temperatures ($p<0.05$), as could be seen in Table 6. However, there were no significant differences of the degradation rate constants among the other groups. At the same level of PVP K30 amount, the microspheres spray dried at the inlet air temperature of 55 ^{o}C shown a lowest degradation rate kinetics, however, it was not significantly different from the inlet air temperature of 65

and 75 ^{o}C. There may be some more explanations to these observations. The microsphere size, as shown in Table 2, might play some role on the degradation rate. The tendency of increased degradation rate could be related to the decreased of particle size. Therefore, further investigation on the effect of microsphere particle size was carried out.

3.2 Effects of microsphere particle size

The spray dried microspheres containing nifedipine:Eudragit RS100:PVP K30 mixing ratio of 1:2:8 was selected as the model formulation in this investigation since it consisted of both carriers, and gave a good yield and optimal drug release in the previous study (Sinsuebpol, 1999).

In order to attain the microspheres of varied particle sizes, the spraying process parameters were modified, e.g., spray concentration and inlet air temperature. The systems used and microsphere sizes are shown in Table 7. Four particle sizes ranged from 6.80 to 41.52 μm. As the particle size increased, the degradation rate constant decreased. To confirm the effect of particle size on degradation rate constant, as shown in Table 7, at the same 65 ^{o}C inlet air temperature, the different microspheres sizes demonstrated the different rate constants.

Table 6 The degradation rate constants and calculated $t_{90\%}$ of nifedipine spray dried microspheres containing varied amounts of PVP K30 in combined polymers and prepared using varied inlet air temperatures.

Inlet air temperature (^{o}C)	PVP K30 content in combined polymers (%)	$k \times 10^{3}$ (h^{-1}) *	$t_{90\%} \times 10^{2}$ (h)
55 ^{o}C	0	111.3	94.7
	20	119.0	88.5
	50	117.8	89.4
	80	119.3	88.3
	100	119.6	88.1
65 ^{o}C	0	116.1	90.7
	20	119.9	87.9
	50	120.2	87.7
	80	119.9	87.9
	100	120.1	87.7
75 ^{o}C	0	115.9	90.9
	20	120.3	87.6
	50	120.7	87.3
	80	119.9	87.9
	100	120.7	87.3

* determined from triplicate samples

Table 7 The degradation rate constants of nifedipine spray dried microspheres obtained from four particle sizes.

Inlet air temperature (^{o}C)	Spray conc. (% w/v)	Pump setting (ml/min)	Spray flow (NL/h)	Median diameter (μm)	Degradation rate constant, k x 10^{3} (h^{-1})*
85	1	10	700	6.35	121.9
65	5	5	600	14.50	119.9
65	20	5	700	25.27	103.6
55	40	5	300	38.31	87.2

* determined from triplicate samples

From two-way ANOVA, at significant level of 0.05, it was found that degradation rate constants were significantly different between the four particle sizes ($p<0.05$) (Table E3). As shown in Figure 10, the degradation rate constant increased with the decreased microspheres size. This might be attributed to the effect of surface area. It is known that photodegradation occurs at the surface of solids (Zhang, 1995). Therefore, the small particles will have higher photodegradation rate than the large particles due to their larger surface area for light exposure. This result consisted to that reported by Teraoka, Otsuka and Matsuda (1999). In their report, the increase in the pure nifedipine powder particle size resulted in the decrease of the degradation rate constant.

3.3 Effect of drug-polymer ratio

In order to investigate the effect of drug concentration and the type of polymer, microspheres of nifedipine with single polymers:Eudragit RS100 or PVP K30 of 1:1 (50% nifedipine), 1:3 (25% nifedipine), 1:5 (16.7% nifedipine) and 1:10 (9.1% nifedipine) were prepared.

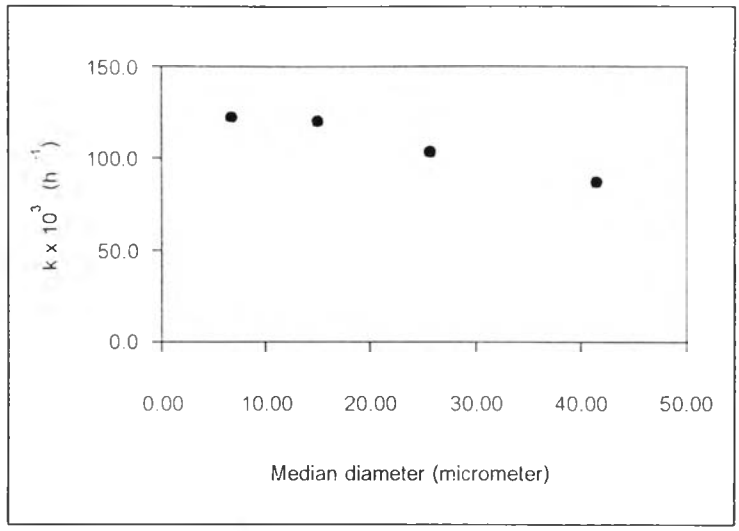

Figure 10 Median diameter and degradation rate constant of 1:2:8 nifedipine:Eudragit RS100:PVP K30 microsphere.

The degradation rate constants of nifedipine microspheres of 1:1, 1:3, 1:5 and 1:10 mixing ratios were 121.1 x 10^{-3}, 120.6 x 10^{-3}, 119.1 x 10^{-3} and 116.1 x 10^{-3} h^{-1}, respectively in Eudragit RS100 system, and were 122.9 x 10^{-3}, 121.4 x 10^{-3}, 128.0 x 10^{-3} and 120.1 x 10^{-3} h^{-1} in PVP K30 system. It can be seen from Figure 11 that the degradation rate constant decreased with the increase of polymer mixing ratio and was higher in the PVP K30 system at the same mixing ratio than that in the Eudragit RS100 system. This might be suggested that as the nifedipine content in the microspheres increased, the reaction site on the particle surface exposed directly and the site inside the particle unexposed directly to the light increased. The environmental nature of the polymer surrounding nifedipine also showed an influence on the degradation rate. The accelerating effect of nifedipine concentration by PVP K30 (hydrophilic polymer) was more pronounced and greater than by Eudragit RS100 (hydrophobic polymer).

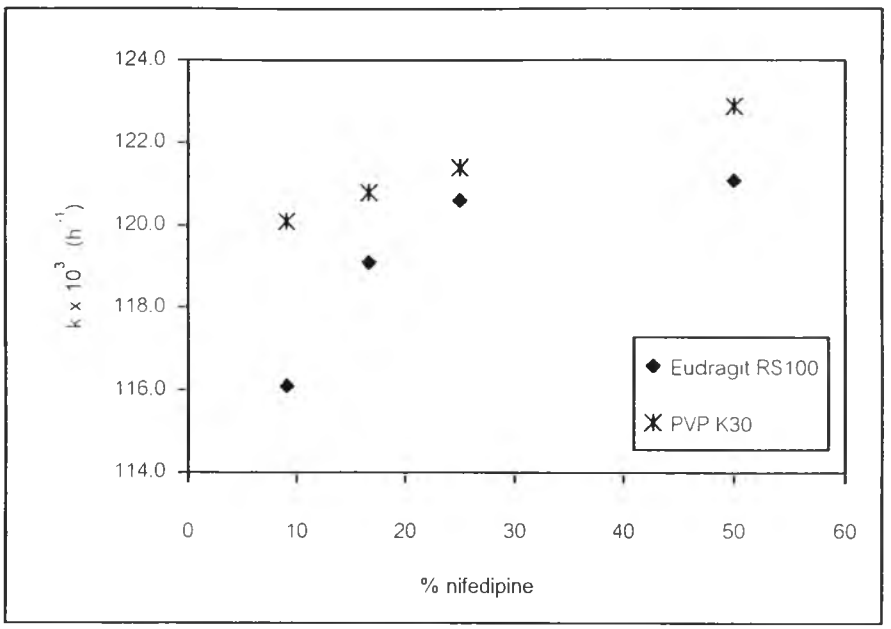

Figure 11 Nifedipine content and degradation rate constant of varied formula of nifedipine microspheres.

Other additional explanation could also extend to the effect of particle size as well. To explore the relationship of particle size and degradation rate constant, the plot was performed as shown in Figure 12. The spray solution of lower polymer mixing ratio gave lower viscosity and without doubt, yield smaller microsphere particle size. As in previous study, the effect of PVP K30 content, nifedipine with PVP K30 microsphere particle size was smaller than that of with Eudragit RS100.

However, from Post Hoc comparison test (Tables E3-E4), the 1:1 ratio gave significantly higher rate constant than other ratios (except 1:5) whereas the 1:10 gave the significantly lower rate constant ($p<0.05$) in the Eudragit system. The similar results were obtained in the PVP K30 system. The 1:1 ratio gave the significantly higher rate constant and the 1:10 gave the significantly lower rate constant (except 1:3) ($p<0.05$).

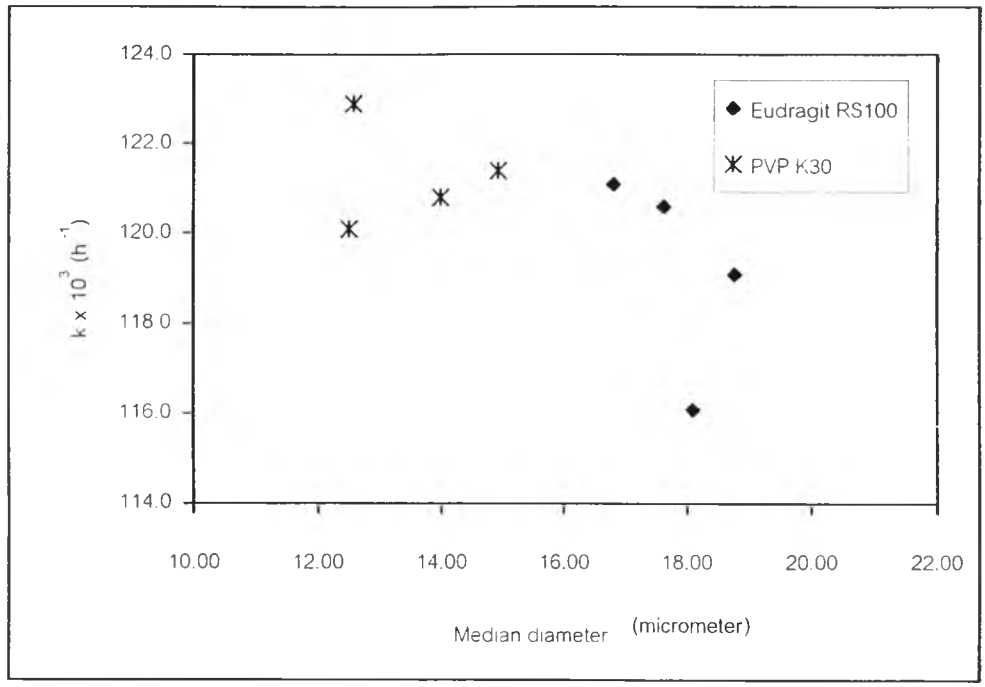

Figure 12 Median diameter versus degradation rate constant of nifedipine microsphere.

3.4 Effect of light intensity

The light intensity in the study was determined by the lux meter, a radiometer used in photometry for measuring the output of a photon source or incident radiant power in the visible region of the electromagnetic spectrum (Tonnesen, 1996). The lux is the power per unit area, expressed as lumen m^{-2} or watt m^{-2}, for irradiance or illuminance.

The four different light intensities were obtained by varying number of fluorescent tubes and distance between samples and light source. The results showed that degradation of nifedipine, a photolabile drug, was markedly effected by the irradiation intensity. The degradation rate constants was 75.7×10^{-3}, 90.8×10^{-3},

119.9×10^{3} and 158.3×10^{-3} h^{-1} from the exposure of 400, 800, 1200 and 2000 lux light intensity, respectively. As it could be seen in Figure 13, the degradation rate constant increased with the increased intensity. This gave the same result as some previous reports (Majeed et al., 1987; Matsuura, Imaizumi, and Sugiyama, 1990).

As the intensity or the light incident upon the microsphere sample increased, the number of photons of a particular wavelength acrossing a unit area in a unit time increased, consequently the energy (*E*) increased. This was demonstrated from the Planck equation

$$E = h\nu$$

Where *h* is Planck' s constant and ν the frequency of light

In photochemical reaction, the photon which is absorbed by a molecule provides the energy to raise the molecule to its excited or reactive state from which the degradation products are formed.

From two-way ANOVA, at significant level of 0.05, it was found that degradation rates were significantly different between the four light intensities ($p<0.05$) as shown in Table E6.

The plot of degradation rate constant against the illuminance was investigated as shown in Figure 13. The plot conformed closely to a linear relationship ($R^2 = 0.9913$).

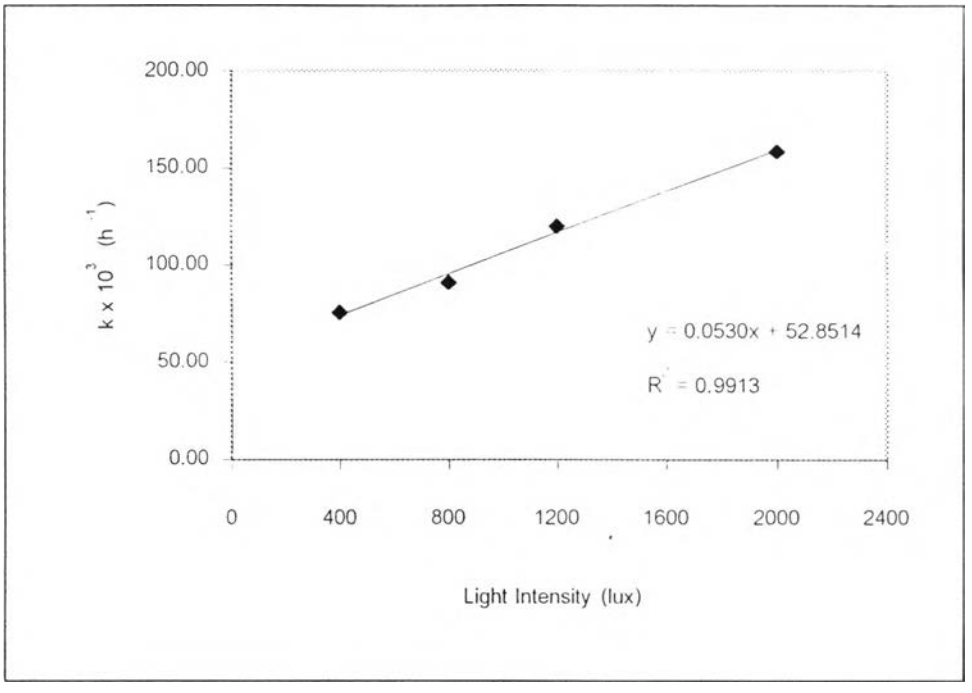

Figure 13 Plot of the degradation rate constant versus the illuminance

The results suggested that it might be possible to predict the photostability under any illumination condition from the plot under accelerated illumination testing. From the plot in Figure 13, the degradation rate constant was predicted to be 7.9 x 10^{-3} h^{-1} and the $t_{90\%}$ (time for 10% photodegradation) for the illuminance at 500 lux which was recommended as the standard illuminance was calculated to be 132.8 h.

3.5 Effect of UV absorbers and antioxidants

Photostabilization of nifedipine in solution state

The concentrations of UV absorbers used in this study were 2, 4, 8, and 16 mg% which were 1, 2, 4, and 8 times of nifedipine concentration. The concentrations of

the antioxidant, sodium bisulfite, were 0.05, 0.1, 0.5, and 1% that were usual concentrations added in dosage forms. The solvent used in the system was 1:1 mixture of methanol and water since nifedipine is practically insoluble in water whereas most UV absorbers (except curcumin) and antioxidants are soluble in water.

From Table 8, it was clearly shown that certain UV absorbers exhibited the photostabilization of nifedipine solution. It was found that in all concentrations of each UV absorber, there were significant differences between control, 2 mg% nifedipine solution, and all experimental groups with UV absorber ($p<0.05$). The protection power increased with the concentration of UV absorbers increased. At the same concentration level, the protection power of curcumin is higher than tartrazine and sunset yellow. This might be attributed to the absorption spectrum of each substance (Thoma, and Klimek, 1991). From Figures 14-19, it can be seen that the absorption spectrum of curcumin was almost covered that of nifedipine while tartrazine and sunset yellow partially covered. However, in case of curcumin crude extract, the protection power was only marginally different from that of tartrazine. This might be due to the less amount of curcumin in the crude extract.

Table 8 The degradation rate constants, protection power and $t_{90\%}$ of 2 mg% nifedipine solution with UV absorbers and antioxidant.

UV absorber/ Antioxidant	Concentration	k x 10^3 (min^{-1}) *	Protection Power [b]	$t_{90\%}$ (min)
Control [a]	-	25.5	1.0	4.1
Curcumin	2 mg%	4.5	5.7	23.4
	4 mg%	2.5	10.2	42.1
	8 mg%	1.6	15.9	65.9
	16 mg%	1.0	25.5	105.4
Curcumin Ext.	2 mg%	15.4	1.7	6.8
	4 mg%	9.6	2.7	11.0
	8 mg%	6.0	4.3	17.6
	16 mg%	3.3	7.7	31.9
Tartrazine	2 mg%	15.2	1.7	6.9
	4 mg%	10.5	2.4	10.0
	8 mg%	6.4	4.0	16.5
	16 mg%	3.8	6.7	27.7
Sunset Yellow	2 mg%	14.4	1.8	7.3
	4 mg%	6.2	4.1	17.0
	8 mg%	5.7	4.5	18.5
	16 mg%	2.6	9.8	40.5
Sod. Bisulfite	0.05 %	25.7	1.0	4.1
	0.1 %	24.5	1.0	4.3
	0.5 %	21.6	1.2	4.9
	1 %	19.9	1.3	5.3

[a] nifedipine 2 mg% solution

[b] determined from degradation rate constant of control / degradation rate constant of stabilizer

* determined from triplicate samples

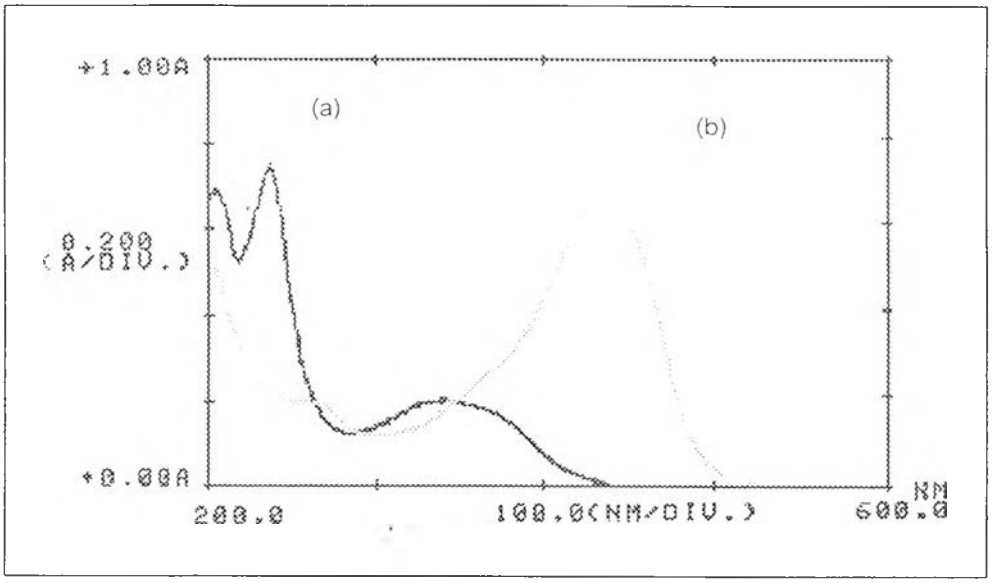

Figure 14 Absorption spectra of nifedipine (a) and curcumin (b) in 1:1 mixture of methanol and water

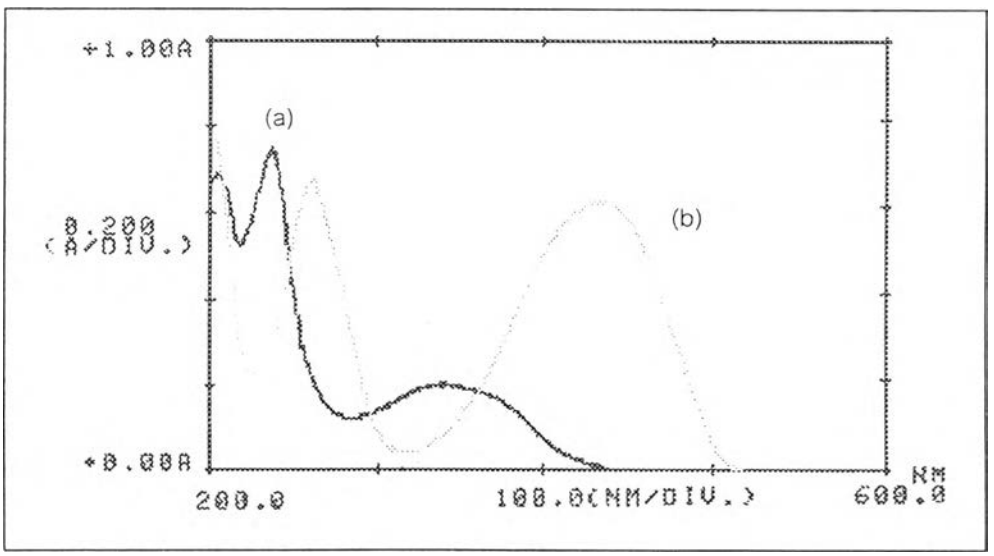

Figure 15 Absorption spectra of nifedipine (a) and tartrazine (b) in 1:1 mixture of methanol and water

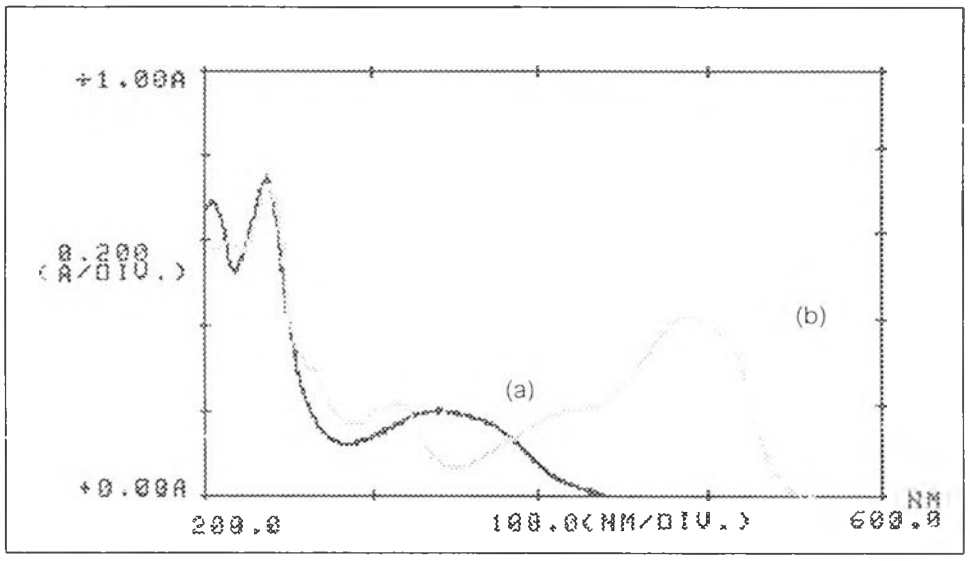

Figure 16 Absorption spectra of nifedipine (a) and sunset yellow (b) in 1:1 mixture of methanol and water

However, in the sodium bisulfite system, there was no significant difference between control group and the nifedipine solution with 0.05% sodium bisulfite ($p=0.271$). Furthermore, the other concentrations of sodium bisulfite added, 0.1, 0.5 and 1%, gave low protection powers as 1.0, 1.2 and 1.3, respectively. This might indicate that the use of antioxidant could not stabilize nifedipine. Therefore, curcumin was selected into further investigations.

Photostabilization of nifedipine in solid state

To investigate the effect of UV absorbers on solid state, nifedipine spray dried microspheres containing 1:2:8:4 mixing ratio nifedipine : Eudragit RS100 : PVP K30 : curcumin were prepared using the same spray drying conditions as control, nifedipine : Eudragit RS100 : PVP K30 ratio of 1:2:8. Even the concentration of curcumin that gave the highest protection power of 25.5 was 16 mg%, the

concentration of 8 mg% that gave slightly less protection power of 15.9 was chosen (Table 8). This was based on the speculation that degradation in solid-state might be less than in solution state. Thus, the curcumin concentration as high as 4 times of nifedipine, was added into the microspheres.

It was noteworthy that the nifedipine microspheres containing curcumin did not show photodegradation through the time of study, 360 hours ($k = 1.4 \times 10^{-3}$ h^{-1}) as shown in Figure 17. As compared with the unstabilized microspheres, nifedipine degraded very quickly with the degradation rate of 119.9×10^{-3} h^{-1}. Moreover, the degradation rate constant of the microspheres with curcumin was not different from the microspheres without curcumin that kept unexposed to light ($p=0.798$). Therefore, it was clear that addition of curcumin at an appropriate concentration exhibited the photoprotection for nifedipine in solid state.

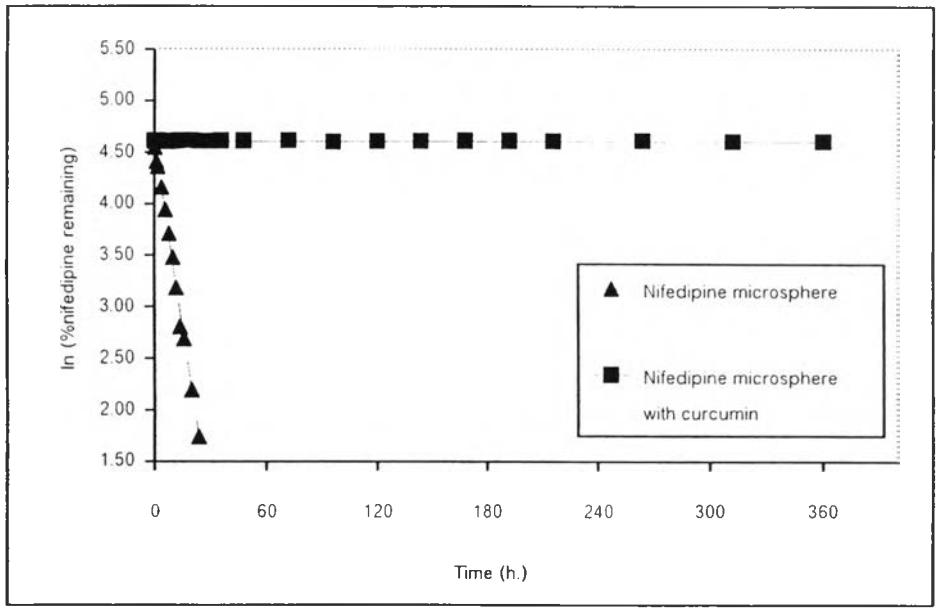

Figure 17 Photodegradation profile of 1:2:8:4 nifedipine : Eudragit RS100 : PVP K30 : Curcumin spray dried microspheres compared with 1:2:8 nifedipine : Eudragit RS100 : PVP K30

4. Effect of relative humidity on nifedipine microsphere stability

4.1 Moisture uptake study

The effect of humidity were carried out by the determination of the water uptake by nifedipine microspheres under varied %relative humidity (%RH). The relative humidities were provided by various saturated salt solutions at 40 ^{o}C. The moisture uptake depended on the %RH and on the PVP K30 content in the microspheres. At the high %RH of 75% and 96%, the water uptake increased very quickly and reach adsorption equilibrium (Figure 18). The water uptake increased significantly with the PVP K30 content increase (Figure 19). This might be due to the hydrophilicity of the polymer and thus hygroscopic to moisture. However, the particle size might relate to the water uptake of the microspheres. From Table 2, as the PVP K30 content in the microspheres increased, the smaller particle size and consequently the larger surface area for moisture adsorption.

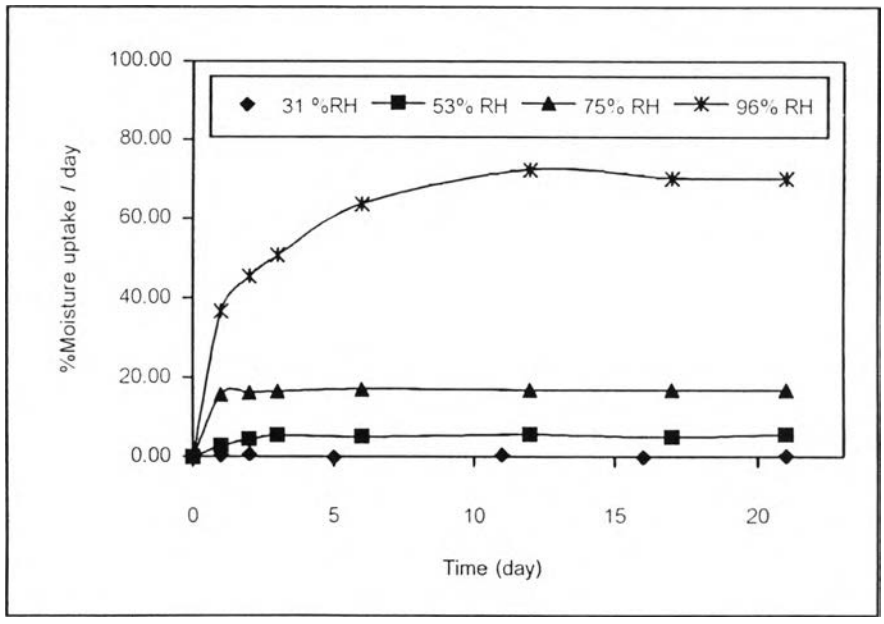

Figure 18 Water uptake profile at varied %RH of 1:0:10 nifedipine:Eudragit RS100: PVP K30 microspheres spray dried at inlet air temperature 65 ^{o}C.

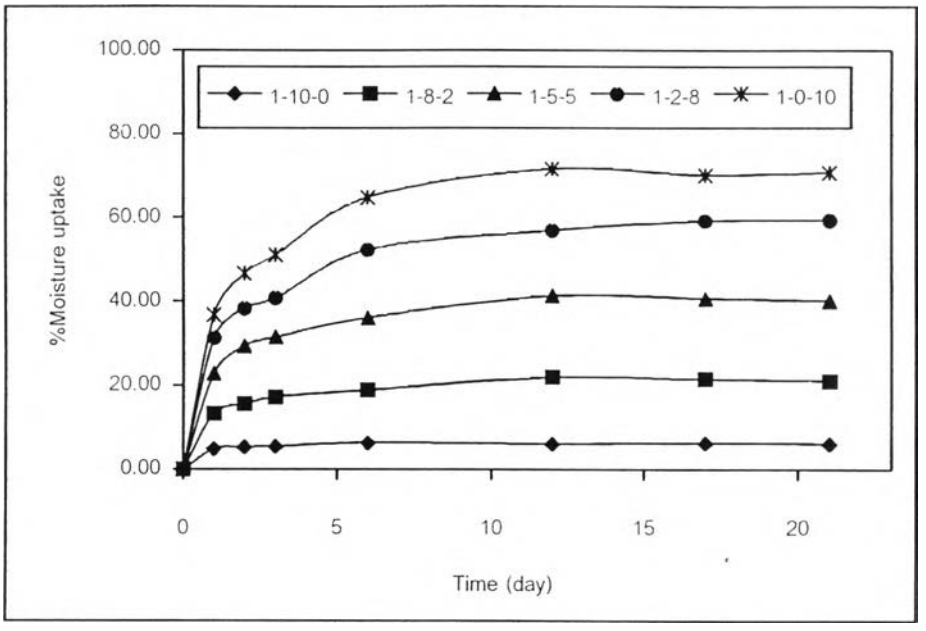

Figure 19 Water uptake profile at 96 %RH of varied formula of nifedipine microspheres spray dried at inlet air temperature 65 ^{o}C.

From the equation modified by Cartensen (Umprayn and Mendes, 1989),

$$W_t - W_o = \delta t$$

where W_t and W_o are the weight of sample at time t and time 0, and δ is the moisture uptake rate. At the rate of moisture uptake $\delta = 0$, the relative humidity of the atmosphere and the relative humidity over the sorbed moisture layer are equal. The intercept at $\delta = 0$ is equal to the critical relative humidity. At this relative humidity or below, no moisture absorption occurs. An example of graph plotted between the relative humidity and moisture uptake rate is shown in Figure 20. From the study, the critical relative humidities of nifedipine microspheres were shown in Table 9. From the non-parametric, Kruskal-Wallis test (Table E9), the results shown that there are no significant difference of the critical relative humidity among

formulas (p = 0.450). However, Table 9 shows roughly that with the PVP K30 content increased, the critical relative humidity decreased. This could be explained by the hygroscopic property of PVP K30 (Windholz, 1983).

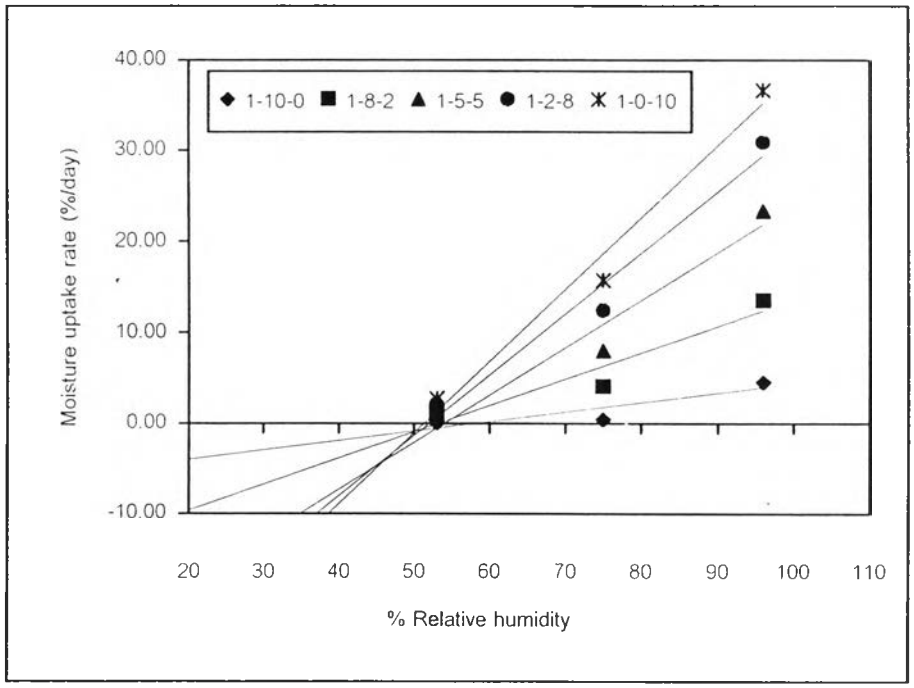

Figure 20 % Relative humidity and water uptake rate of varied formula of nifedipine microspheres spray dried at inlet air temperature 65 ^{o}C.

Table 9 Critical relative humidity (%RH) of nifedipine spray dried microspheres.

Nifedipine : Eudragit RS100 : PVP K30	Critical Relative Humidity (%RH)		
	55 (^{o}C)	65 (^{o}C)	75 (^{o}C)
1:10:0	56.2	61.5	59.7
1:8:2	53.9	55.2	54.4
1:5:5	53.0	55.0	54.7
1:2:8	50.9	52.6	53.1
1:0:10	49.5	51.8	49.7

4.2 Chemical stability study

After storing at the varied relative humidities for 21 days, the samples were analyzed for nifedipine content by the HPLC method. The significant difference between the experimental and the control samples of each formula was compared by the non-parametric, Friedman test (Table E10). The results showed that there was no significant difference between the experimental and the control sample ($p=0.058$). Therefore, the humidity did not effect the chemical stability of nifedipine microspheres. However, the physical instability occurred, especially, the formula of 1:2:8 and 1:0:10 nifedipine:Eudragit RS100:PVP K30 which consisted of high content of hygroscopic polymer, PVP K30 were liquefied at all %RH exposure except those at the 31 %RH.

5. Effect of light, relative humidity and temperature in ambient atmosphere on nifedipine microsphere stability.

5.1 Chemical stability study

In order to obtain ambient condition, the light cabinet doors were left open and the distance between samples and light sources was adjusted to get the light intensity approximated to that of in ambient atmosphere, 1000 lux.

From Post Hoc comparison test, there are no significant difference between the degradation rate constant among all formulas except the experimental group of nifedipine microsphere ($p<0.05$ for all comparisons) (Table E11). This meant that after 360 hours, nifedipine in the microspheres with and without curcumin in the control group and nifedipine amount in the microspheres with curcumin in the experimental group still remained unchanged as the initial time ($k = 0.5 \times 10^{-3}\ h^{-1}$) while the microspheres without curcumin in the experimental group decomposed

with degradation rate constant of 96.6×10^{-3} h^{-1} . This can be concluded that the addition of curcumin into nifedipine microspheres can remarkably protect photodegradation of nifedipine in ambient atmosphere without special protective packaging requirement, such as amber glass containers and aluminum foils.

5.2 Dissolution characteristics of nifedipine microspheres

To determine the physical stability of the microspheres under ambient condition, the release characteristics of the microspheres were focused. The dissolution study of nifedipine microspheres with and without curcumin in the experimental group and control group were performed in the simulated intestinal fluid pH 7.5 for 24 h. An example of the dissolution profile is shown in Figure 21.

To determine the release kinetics, graphs of %nifedipine released versus time, ln(%nifedipine released) versus time, 1/ (%nifedipine released) versus time and %nifedipine released versus square root time were plotted according to zero-order, first-order, second-order and Higuchi equation. Linear regression was used to determine the coefficient of determination (R^2) which were then compared, as shown in Table C5. The results indicated that the plot of %nifedipine released versus square root time gave the highest coefficient of determination. Therefore, the release mechanism of nifedipine from spray dried microspheres followed Higuchi model.

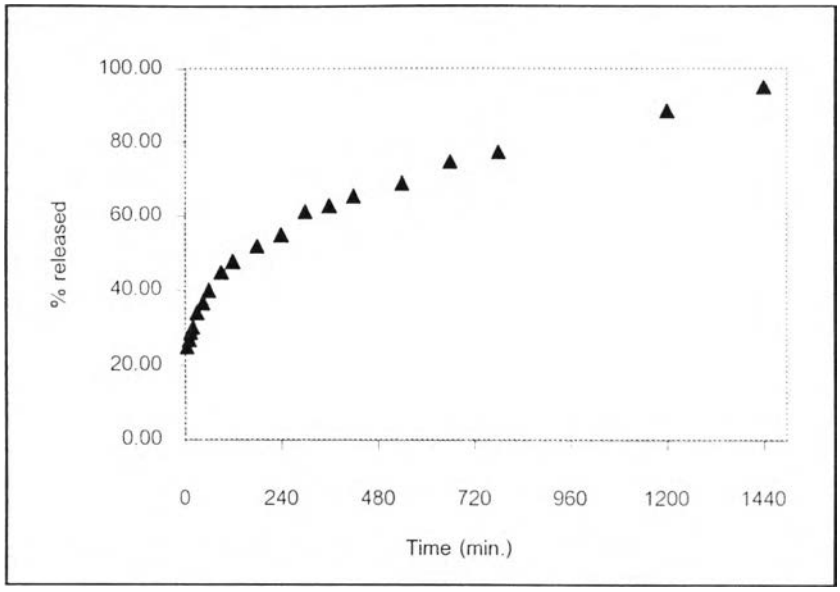

Figure 21 Zero-order plot of dissolution of 1:2:8:4 nifedipine:Eudragit RS100:PVP K30:curcumin spray dried microspheres.

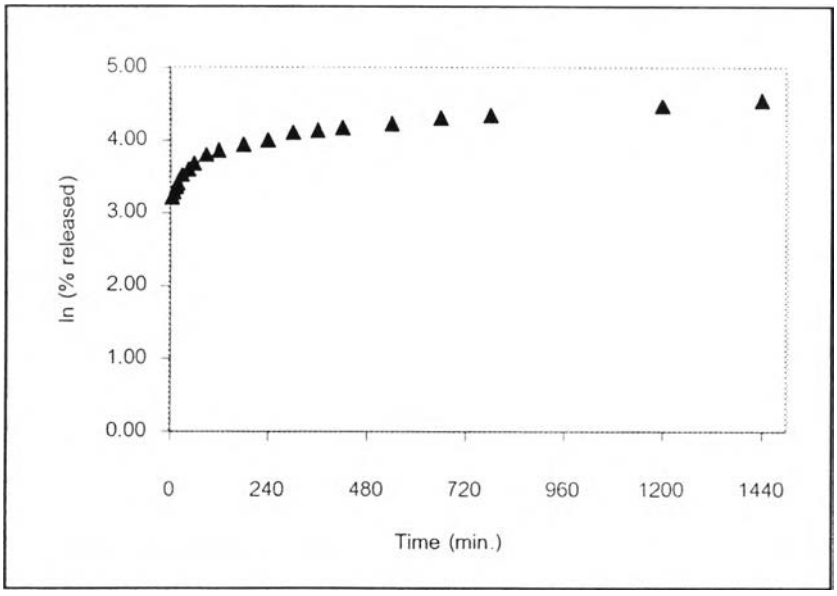

Figure 22 First-order plot of dissolution of 1:2:8:4 nifedipine:Eudragit RS100:PVP K30:curcumin spray dried microspheres.

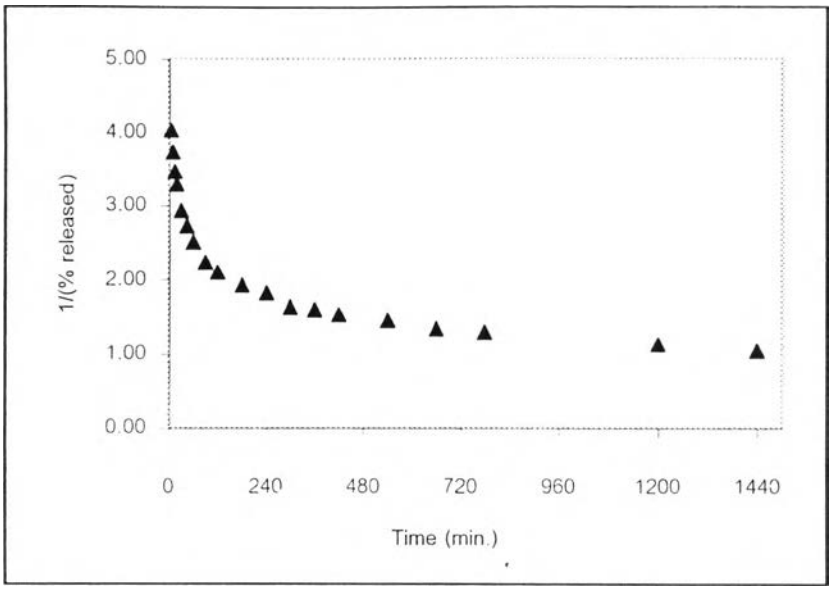

Figure 23 Second-order plot of dissolution of 1:2:8:4 nifedipine:Eudragit RS100:PVP K30:curcumin spray dried microspheres.

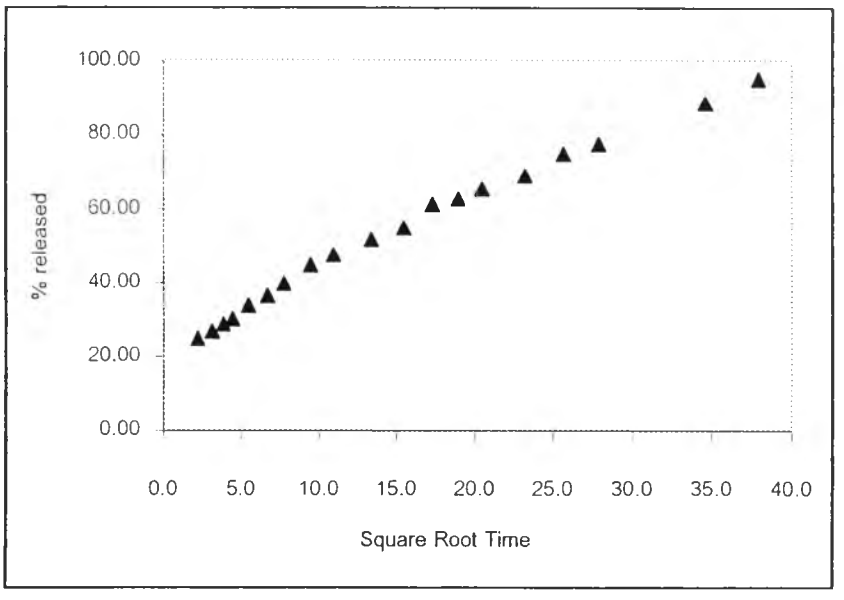

Figure 24 Higuchi plot of dissolution of 1:2:8:4 nifedipine:Eudragit RS100:PVP K30:curcumin spray dried microspheres.

The dissolution profiles of the microspheres with and without curcumin in the experimental and control groups are compared in Figure 25. From two-way ANOVA, at significance level of 0.05, there was no significant difference between the dissolution rate constants of all groups (between the control and the experimental groups p=4.735, between the microspheres with and without curcumin groups p=0.688) (Table E12). The indifferent dissolution rate constants between the microspheres with and without curcumin in the experimental group (exposed to ambient condition) and with microspheres in the control group suggested that nifedipine microspheres were physical stable on the basis of the dissolution characteristics. This was independent on the chemical degradation. This might suggest that the relative humidity of the ambient condition did not effect nifedipine amorphous form. From the previous report (Sinsuebpol, 1999), nifedipine existed in the spray dried microspheres in an amorphous state. Hasegawa, Nakagawa, and Sugimoto (1985) reported that nifedipine and hydroxypropylmethyl-cellulosephthalate, and nifedipine and Eudragit L solid dispersions stored at 40 ^{o}C, 80% RH for 6 months did not change in dissolution behavior. From the x-ray diffraction study in their study, it was found that there were no any sharp peak which attributable to nifedipine crystal in the x-ray diffraction pattern. However, the peaks were found in nifedipine and povidone, the hygroscopic polymer, solid dispersion that the dissolution behavior was found to be decreased.

From the results of the investigation in this section, it might be proposed that the photostabilization of nifedipine in solid-state, e.g. microspheres, was possibly accomplished by the addition of curcumin at an appropriate concentration.

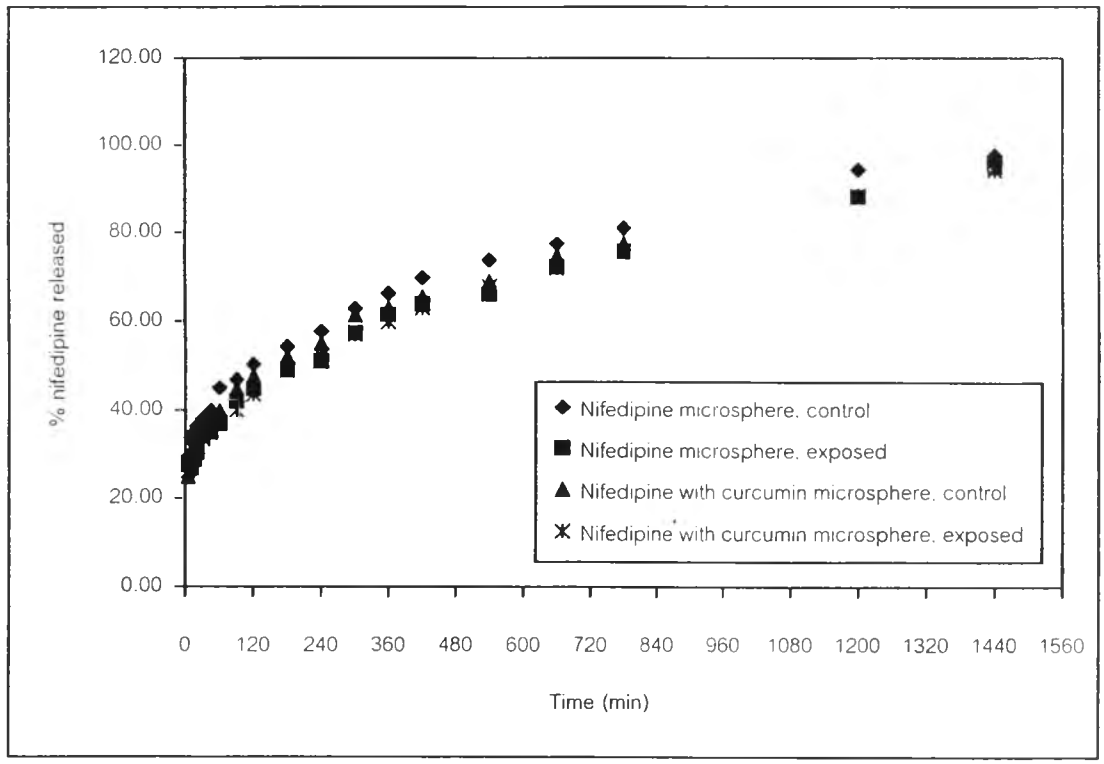

Figure 25 Dissolution profiles of 1:2:8 nifedipine:Eudragit RS100:PVP K30 and 1:2:8:4 nifedipine:Eudragit RS100:PVP K30:curcumin microspheres in the experimental and control groups