Chapter III

Results and Discussion

The results are classified into two main sections according to the procedures described in the previous chapter as follows.

1. Formulation Development and In Vitro Evaluation

1.1 Preparation of Matrices by Wet Granulation Method

In the preparation of matrices, Methocel E4M as dried powder were mixed with theophylline and/or lactose by geometric dilution and then absolute ethanol was added to make suitable wet mass. It should be noted that Methocel E4M as aqueous solution was not selected to be binder because of two principal reasons. Firstly, its high viscosity may make such sticky mass that the drug could not be regularly spreaded in the matrices. this result directly affected both the uniformity of drug content and drug release. Another reason was that it was much more easier to vary the amount of polymer in the formulation as dried powder than to prepare various concentrations of polymer solution. However, the production of wet granules using ethanol had to be done in a hurry in order to avoid too much fines from the harder mass caused by ethanol evaporation.On the contrary, the advantage of ethanol as a granulation agent was that the problem of overwetting could be easily solved by allowing the ethanol to evaporate while kneading the mass simultaneously.

1.2 Physical Properties of Granules

1.2.1 Moisture Content : There was a little difference in moisture content among six formulations as shown in Table 5. Tablets with 40 % lactose (formulation 6), however, had the highest moisture of all.

It should be noted that the moisture content of all granulations, with and without lactose, was less than 1 % which was quite low.

1.2.2 Particle Size Distribution : Among formulations 4, 5, and 6, the percent weight of granules whose size was > 850 μm tended to decrease while that of granules whose size was < 150 μm was likely to change in the reverse order ; as the amount of lactose increased (Table 6 and Figure 2).

The possible explanation was that an increase of lactose in the formulation caused consequent dilution of Methocel E4M, which equally weakened the binding force within granules. The larger particles, therefore, tended to be easily broken into fines.

1.2.3 Angle of Repose, Bulk and Tap Density, and Compressibility: The difference among all formulations did not seem to have the effect on these three parameters. As shown in Table 7, the repose angle of 35.4 - 37.1 was almost the same while the bulk density, tap density and compressibility showed only a little difference.

Table 5 Moisture Content of Theophylline Granules Prepared by Wet Granulation Method

Formulation	% Moisture Content (S.D.)*
Without Lactose :	
1	0.41 (0.06)
2	0.58 (0.05)
3	0.43 (0.03)
With Lactose :	
4	0.55 (0.06)
5	0.57 (0.14)
6	0.96 (0.22)

* average from three determinations

Table 6 Particle Size Distribution of Granules Prepared by Wet Granulation Method

Formulation	% Weight Retained on Sieve Series					
	Pan	150 μm	180 μm	250 μm	425 μm	850 μm
Without Lactose						
1	6.0	2.6	3.8	7.5	18.5	58.6
2	4.7	3.5	4.6	8.8	18.4	55.8
3	6.4	5.4	5.0	9.8	22.3	47.6
With Lactose						
4	9.2	4.3	5.0	10.8	22.8	46.0
5	13.8	4.4	5.4	10.7	24.5	38.0
6	15.4	5.3	6.0	12.5	26.8	31.5

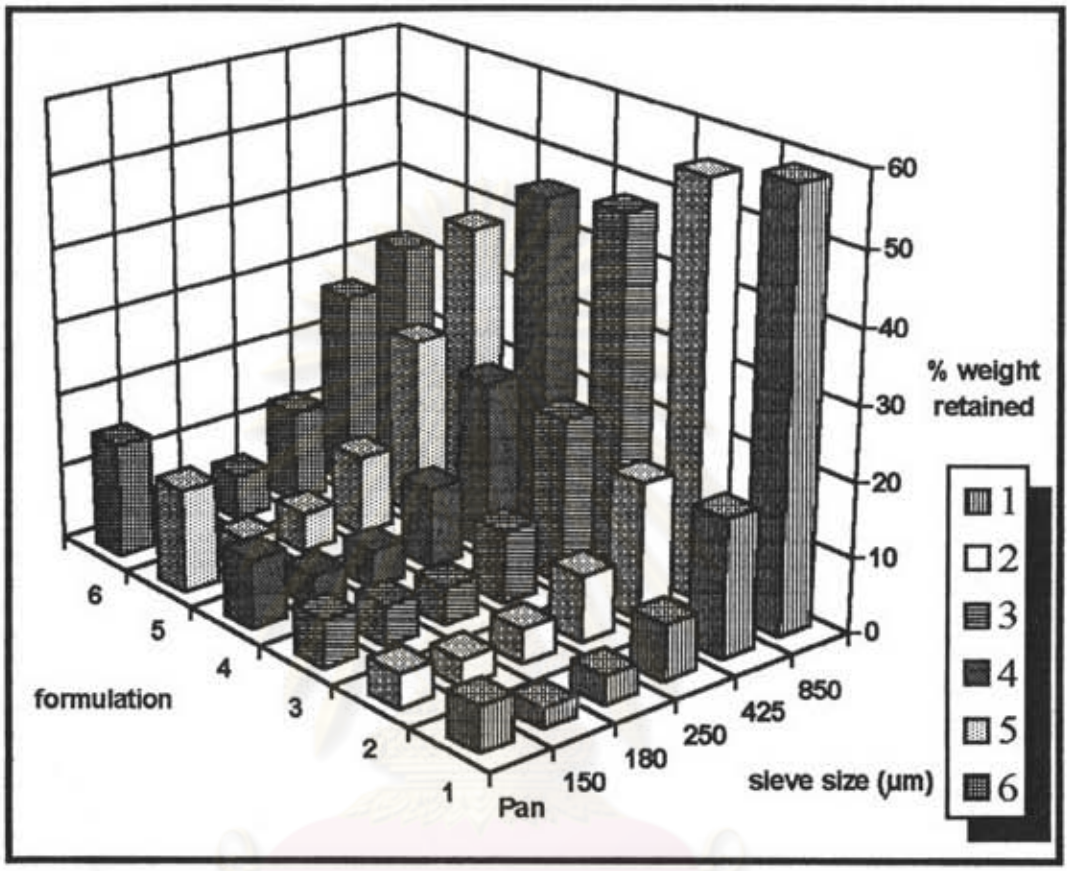

Figure 2 Particle Size Distribution of Theophylline Granules Prepared by Wet Granulation Method

Table 7 Angle of Repose, Bulk Density, Tap Density and Compressibility of Theophylline Granules Prepared by Wet Granulation Method

Formulation	Angle of Repose(°)	Bulk Density (g/mL)	Tap Density (g/mL)	Compressibility (%)
Without Lactose				
1	35.5	0.51	0.59	13.56
2	35.4	0.46	0.53	13.21
3	37.1	0.45	0.52	13.46
With Lactose				
4	36.0	0.44	0.52	15.38
5	36.0	0.43	0.51	15.69
6	36.3	0.45	0.51	11.76

This indicated that the method of preparation could produce the same proportion of spherical and irregular-shaped granules which accordingly produced the same flowability. A little difference in particle size distribution of each formulation may be supposed to give the nearly similar bulk and tap density and also compressibility which was derived from both parameters.

1.2.4 Theophylline Content : Some variations in the percentage of drug content was evident among each formulation (Table 8). The low standard deviation, however, exhibited the uniformity of drug distribution in the granules.

1.3 Physical Properties of Tablets

1.3.1 Hardness and Disintegration Time : The average values of hardness are mostly ranging from about 9.90 - 11.05 kp and the disintegration times of all tablets are more than 120 minutes (Table 9).

In this study, the hardness of tablet was controlled at around 10 kp by adjustment of compression pressure to avoid the effect of this variable on the release of drug from matrices. In case of disintegration time, it could be explained by the mechanism of HPMC in controlling drug release. On exposure to the disintegration fluid, tablet surface became wet and the polymer started to partially hydrate to form a gel

Table 8 Theophylline Content of Granules Prepared by Wet Granulation Method

Formulation	% Drug Content *			
	1	2	3	Mean(S.D.)
Without Lactose				
1	99.07	101.40	102.30	100.92(1.67)
2	102.27	101.00	102.10	101.79(0.69)
3	101.99	101.89	96.14	100.01(3.35)
With Lactose				
4	102.50	102.03	102.36	102.30(0.24)
5	102.60	104.37	104.17	103.71(0.97)
6	103.53	104.43	105.47	104.48(0.97)

*average from three determinations

Table 9 Physical Properties of Theophylline Sustained-releaseTablets Prepared by Wet Granulation Method

Formulation	Hardness, kp(SD)*	Disintegration Time**
Without Lactose		
1	10.54 (0.46)	> 120
2	9.90 (0.22)	> 120
3	10.59(0.25)	> 120
With Lactose		
4	11.05 (0.29)	> 120
5	11.01 (0.38)	> 120
6	10.10 (0.30)	> 120

*n = 10

**n = 6

layer. The thickness of this layer was increasing as water permeated into the tablet. Concomitantly the outer layers became fully hydrated and dissolved. Water continued to penetrate towards the tablet core until it has dissolved (Shangraw, 1988 ; Hogan, 1989). By this pattern, the drug slowly dissolved and was then released by diffusion through the gel barrier without disintegration.

1.4 Effect of pH on Dissolution Characteristics

Figures 3 and 4 were the profiles of average percent theophylline dissolved from tablets without lactose in 0.1 N HCl and pH 6.8 phosphate buffer, respectively. It was indicated that all formulations had faster release rate in acidic medium than in pH 6.8 buffer. Drug release from tablets with 5 % Methocel E4M (formulation 1) in 0.1 N HCl was shown to be much faster than that in pH 6.8 buffer. An increase of the polymer to 7 % (formulation 2) could produce additional retarding effect on dissolution rate in both medium. However, the further increase to 10 % polymer (formulation 3) provided more slower release rate only in 0.1 N HCl but had very little effect on drug release in pH 6.8 medium.

This is due to the faster rate of hydration of HPMC in acidic medium than in medium of higher pH which directly affected the rate of drug dissolution. In addition, it was apparent that the effect of pH on drug release related with the quantity of Methocel E4M in the formulation. That was for the tablets with low amount of polymer, the release of

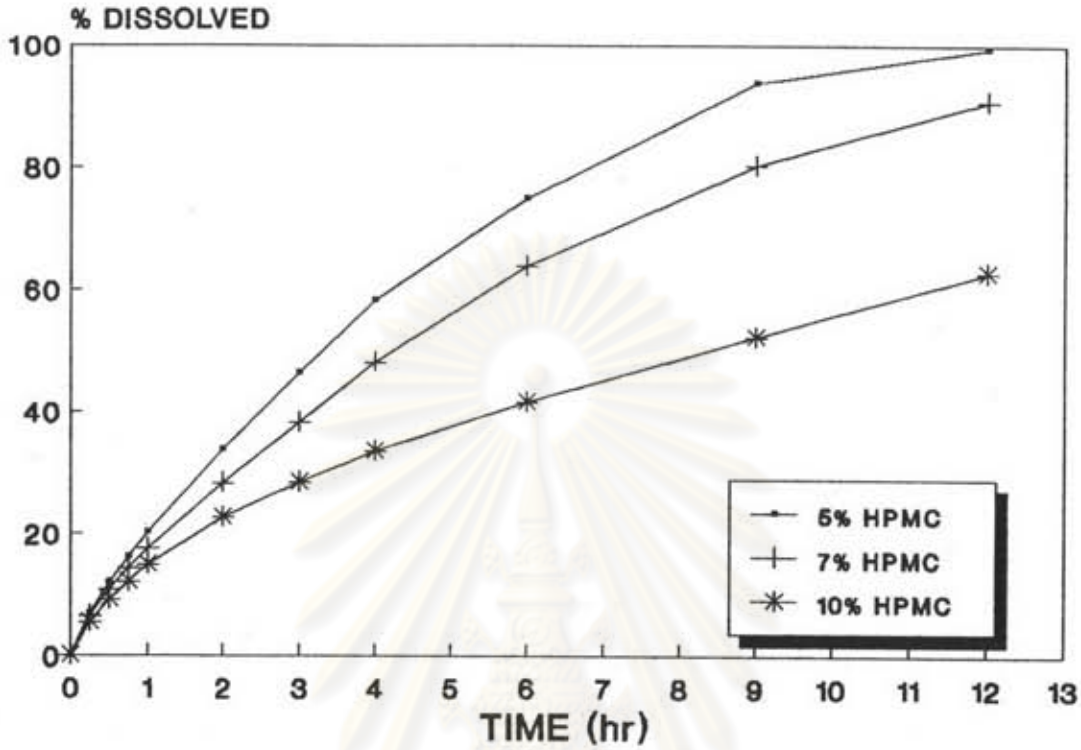

Figure 3 Dissolution Profiles of Theophylline Sustained-release Tablets without Lactose in 0.1 N Hydrochloric Acid

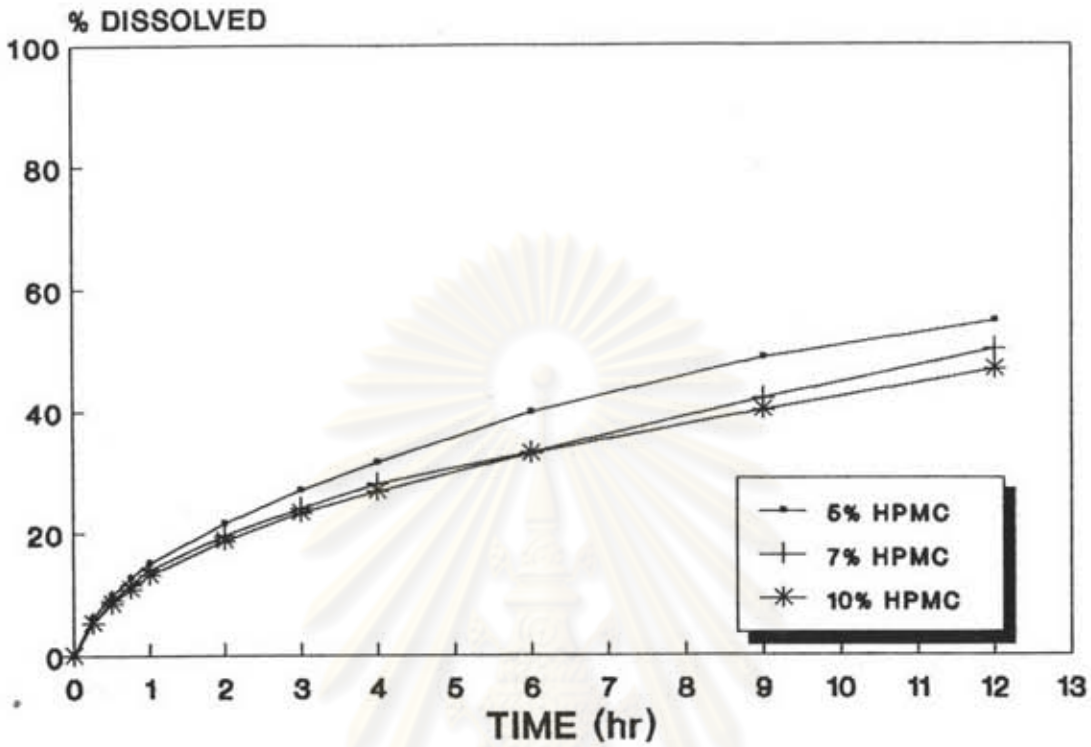

Figure 4 Dissolution Profiles of Theophylline Sustained-release Tablets without Lactose in pH 6.8 Phosphate Buffer

theophylline in pH 6.8 buffer seemed to be slower than in acidic medium. Furthermore, an increase of polymer could slower the release rate in both medium but with a larger degree in 0.1 N HCl. Therefore, it may be stated that the increase of Methocel E4M could reduce the effect of medium pH on drug release which finally gave more similar release patterns in both medium.

1.5 Effect of Lactose on Dissolution Profiles Tested by pH Change Method

From the results in 1.4, formulation 2 (7 % Methocel E4M) was chosen for further development for two reasons . Firstly, formulation 1(5 % Methocel E4M) gave much more drug release in 0.1 N HCl than formulation 2 did, meanwhile their release patterns in pH 6.8 medium were nearly similar. Secondly, although formulation 3 (10 % Methocel E4M) provided the most similar release profiles in both medium, the release rate was too slow.

It was found that the addition of lactose at 20 % initial quantity was able to increase the drug dissolution in both medium (Figure 5) when compared to the formulation without lactose (Figures 3 and 4). In addition, this effect had a greater extent to the release in pH 6.8 medium than in 0.1 N HCl in which drug dissolution slightly increased in the first two hours. The dissolution profiles of tablets with various amounts of lactose obtained by pH change method were shown in Figures 6 to 8. It

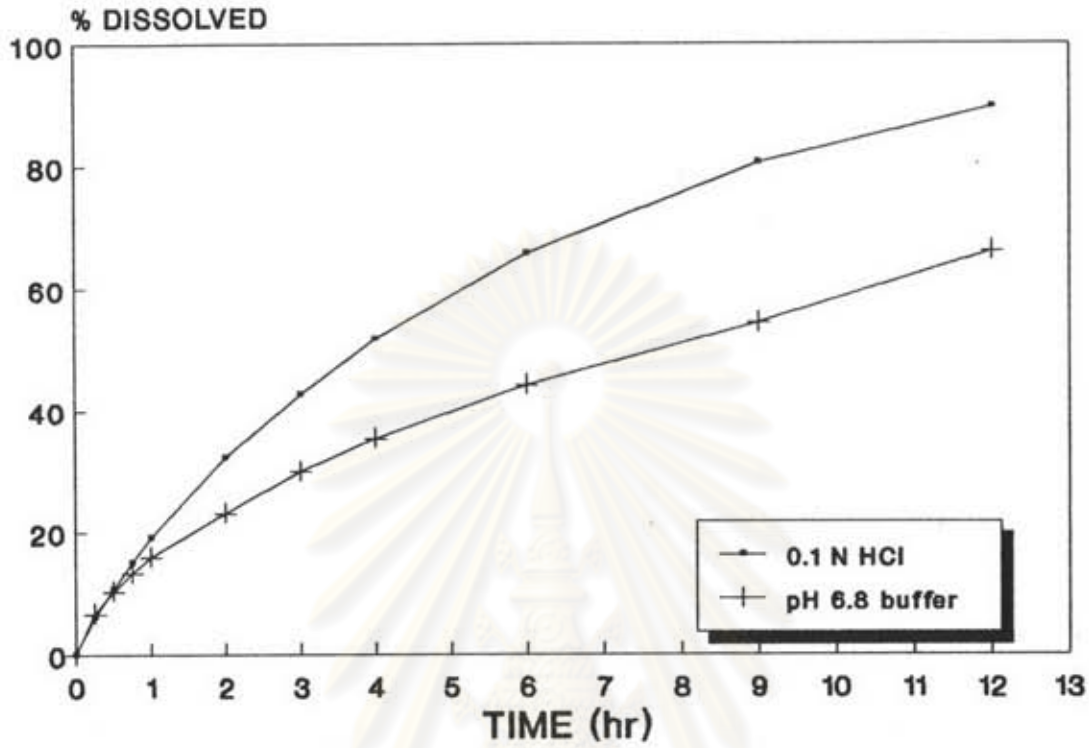

Figure 5 Dissolution Profiles of Theophylline Sustained-release Tablets with 20 % Lactose in 0.1 N Hydrochloric Acid and pH 6.8 Phosphate Buffer

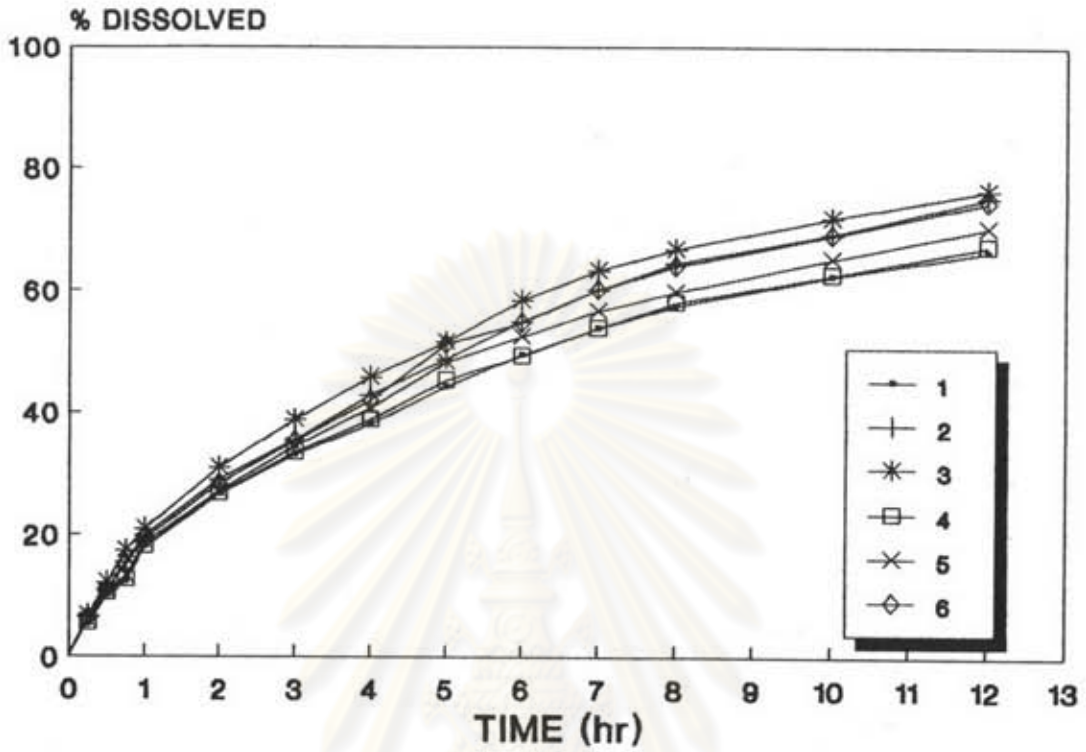

Figure 6 Dissolution Profiles of Six Theophylline Sustained-release Tablets with 20 % Lactose Tested by pH Change Method

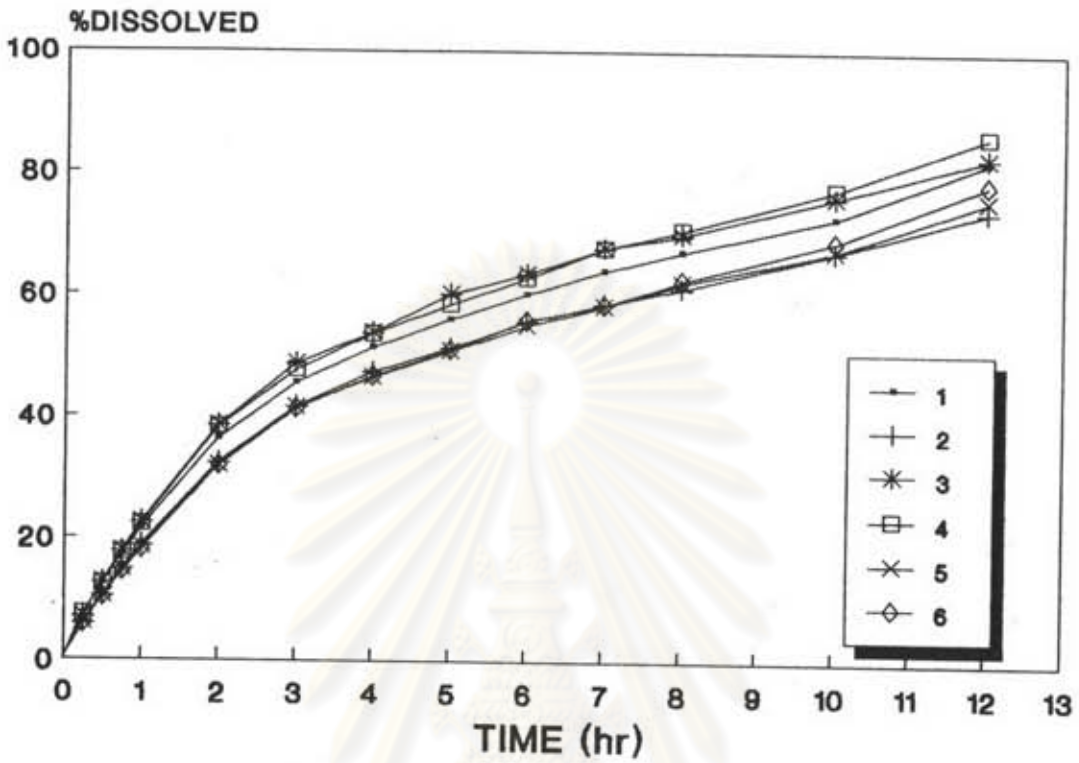

Figure 7 Dissolution Profiles of Six Theophylline Sustained-release Tablets with 30 % Lactose Tested by pH Change Method

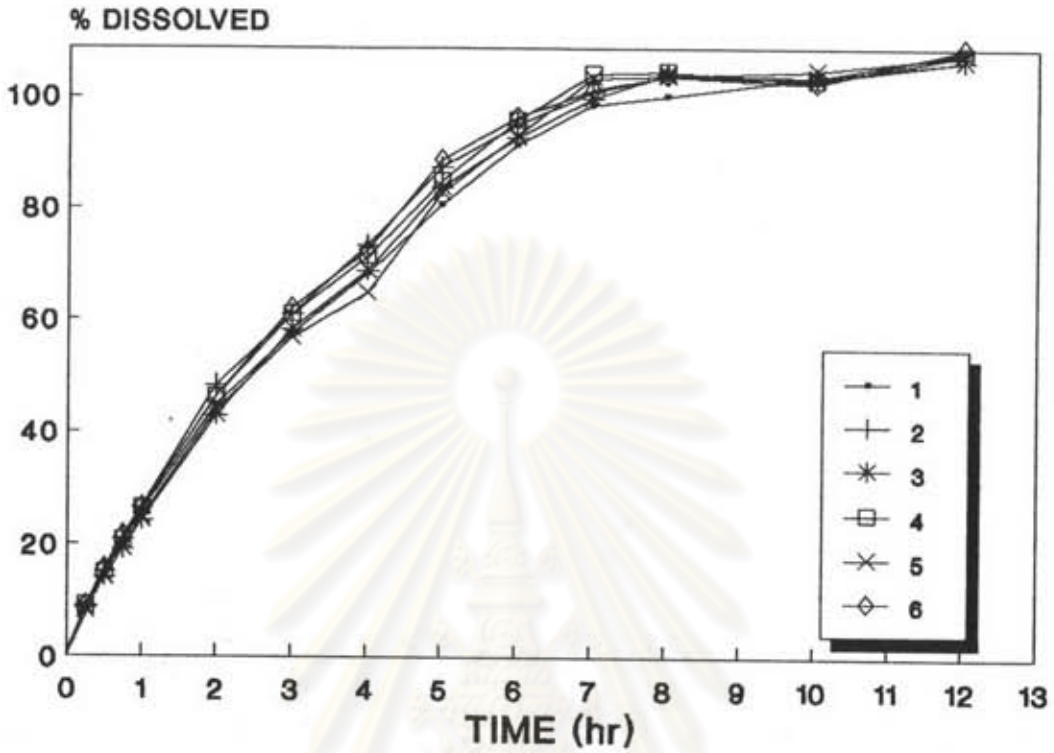

Figure 8 Dissolution Profiles of Six Theophylline Sustained-release Tablets with 40 % Lactose Tested by pH Change Method

should be noted that the dissolution rate was likely to increase in proportion to the percentage of lactose in the formulations. The tablets with 30 % lactose provided about 80 % of theophylline dissolved within 12 hours (Figure 7) while tablets with 20 % lactose yielded less amount within the same time (Figure 6). For 40 % lactose-containing tablets, the release was completely terminated at about 7 hours (Figure 8).

The cause of this finding may be due to the dilution of Methocel E4M caused by the increase of lactose in the formulation. Another possible reason was that water can penetrate into the tablet by the pore occurred after the dissolution of lactose. This channel is helpful to increase the surface area of the tablet and the following result was an increasing rate of drug dissolution (Ford, 1985 ; Sanghavi et al., 1990).

1.6 In Vitro Release Patterns of Theophylline Sustained-release Tablets Prepared by Different Techniques

The dissolution profiles of the experimental sustained-release theophylline tablets (C, D, E) and the commercial products (A, B) , summarized in Table 10, were illustrated in Figures 7, 9 - 10 and Figures 11 - 12, respectively. It was obviously seen that product C that was prepared using spray drying process showed the relatively high degree of uniform drug release of which standard deviation at each sampling time was the lowest among all products tested. This result agreed with that

Table 10 Dissolution Profiles of Five Formulations of Theophylline Sustained-release Tablets Tested by pH Change Method

Time	% Drug Dissolved (SD)				
	A	B	C	D	E
0.25	4.76(0.51)	8.08(0.20)	7.32(0.43)	2.77(0.60)	6.52(0.74)
0.50	7.19(0.69)	12.44(0.36)	13.17(0.76)	7.19(1.07)	11.40(1.18)
0.75	9.08(0.89)	15.93(0.28)	18.88(0.93)	11.31(0.73)	15.95(1.65)
1	11.04(0.96)	18.99(0.27)	24.52(0.87)	14.95(0.79)	20.24(2.02)
2	15.26(0.99)	26.70(0.31)	39.82(0.69)	25.95(1.20)	34.77(3.22)
3	17.89(1.17)	34.94(1.33)	46.20(0.84)	34.07(1.23)	44.27(3.30)
4	21.81(2.29)	45.82(2.74)	52.48(0.73)	41.66(1.73)	49.71(3.45)
5	28.12(4.30)	57.81(4.32)	57.43(1.03)	46.86(2.05)	54.55(4.19)
6	35.28(4.76)	68.72(4.99)	61.79(0.73)	51.76(2.93)	58.84(3.87)
7	43.08(5.22)	76.56(6.68)	66.58(0.84)	55.76(2.81)	62.42(4.64)
8	51.24(6.49)	83.70(7.23)	71.11(0.68)	59.09(3.00)	65.59(4.26)
10	65.41(6.20)	91.76(5.65)	76.85(1.06)	65.50(3.91)	71.61(4.44)
12	77.04(6.05)	98.22(2.73)	82.64(0.94)	70.31(3.73)	79.88(4.62)

Where A = Theo-Dur® ; B = Nuelin® ; C, D, and E = Tablets prepared by spray drying, fluidized-bed coating and conventional wet granulation respectively

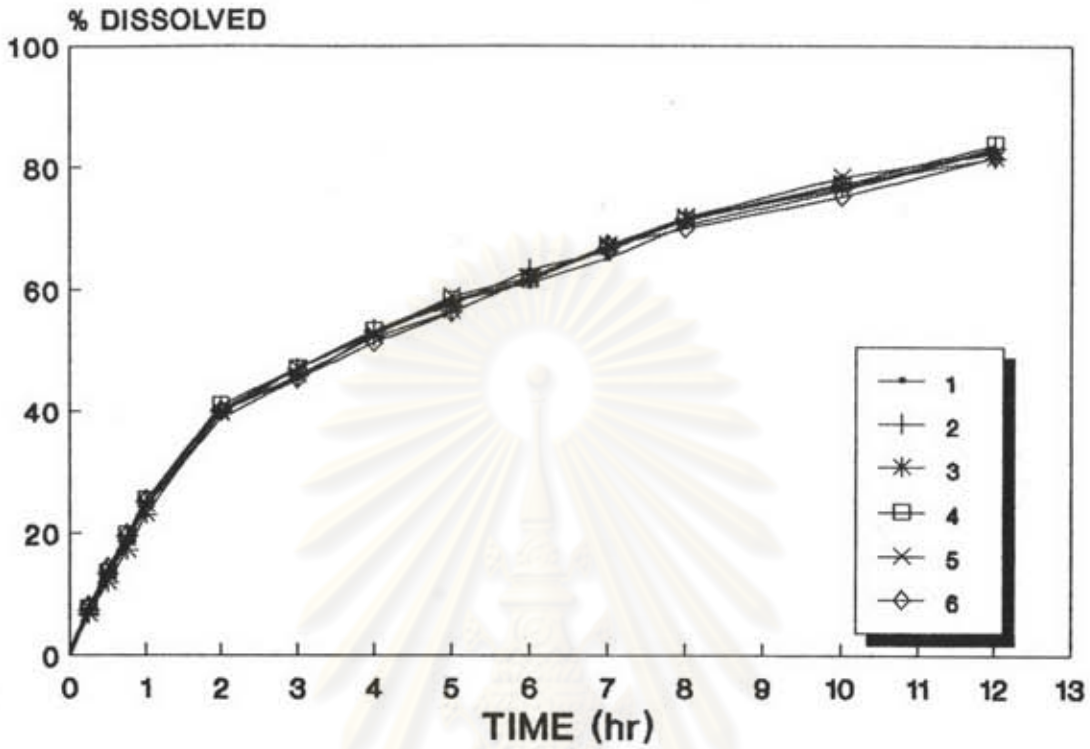

Figure 9 Dissolution Profiles of Six Theophylline Sustained-release Tablets Prepared by Spray Drying Technique (pH Change)

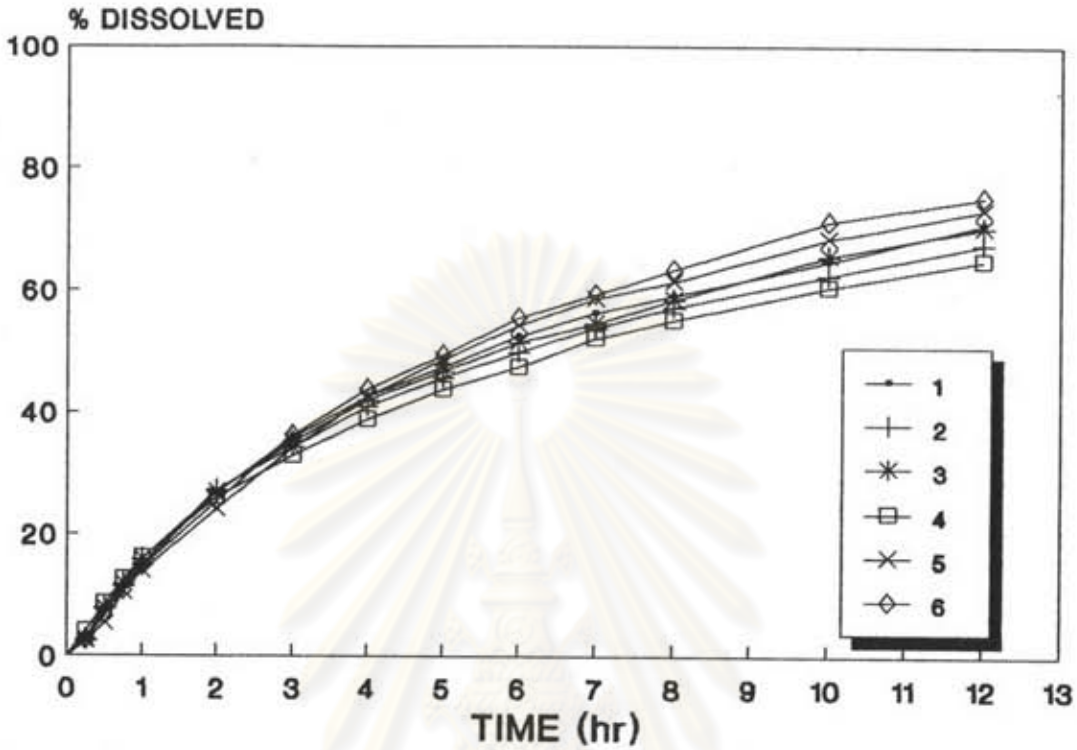

Figure 10 Dissolution Profiles of Six Theophylline Sustained-release Tablets Prepared by Fluidization Coating (pH Change)

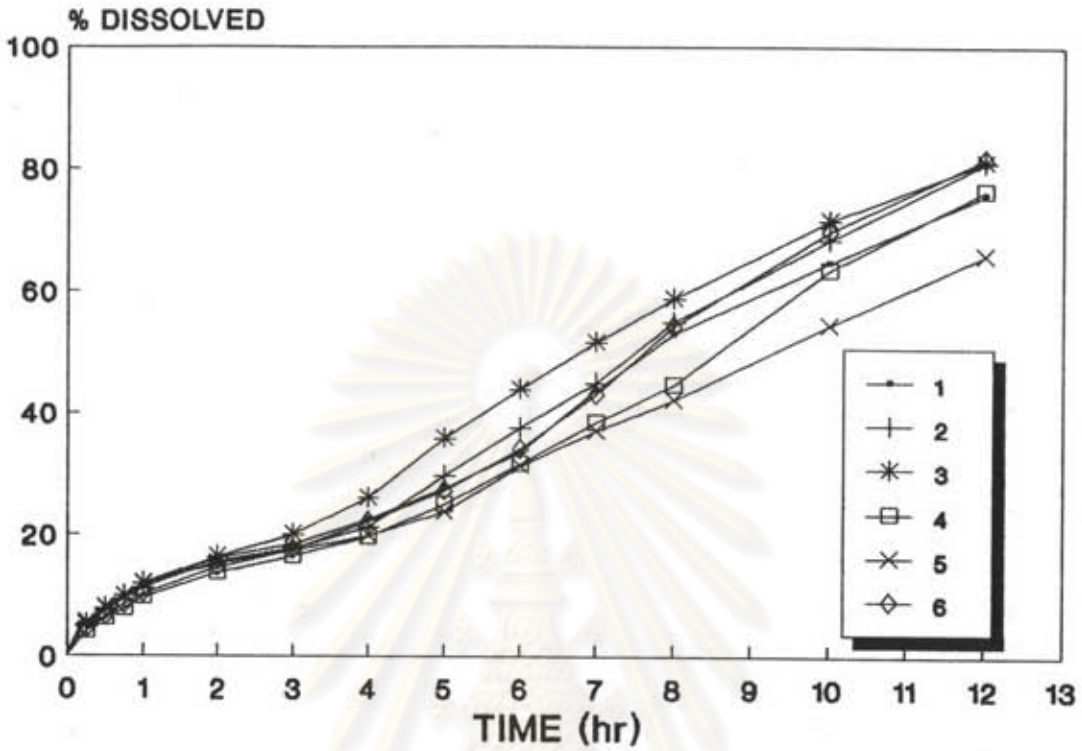

Figure 11 Dissolution Profiles of Six Tablets of Theo-Dur® Tested by pH Change Method

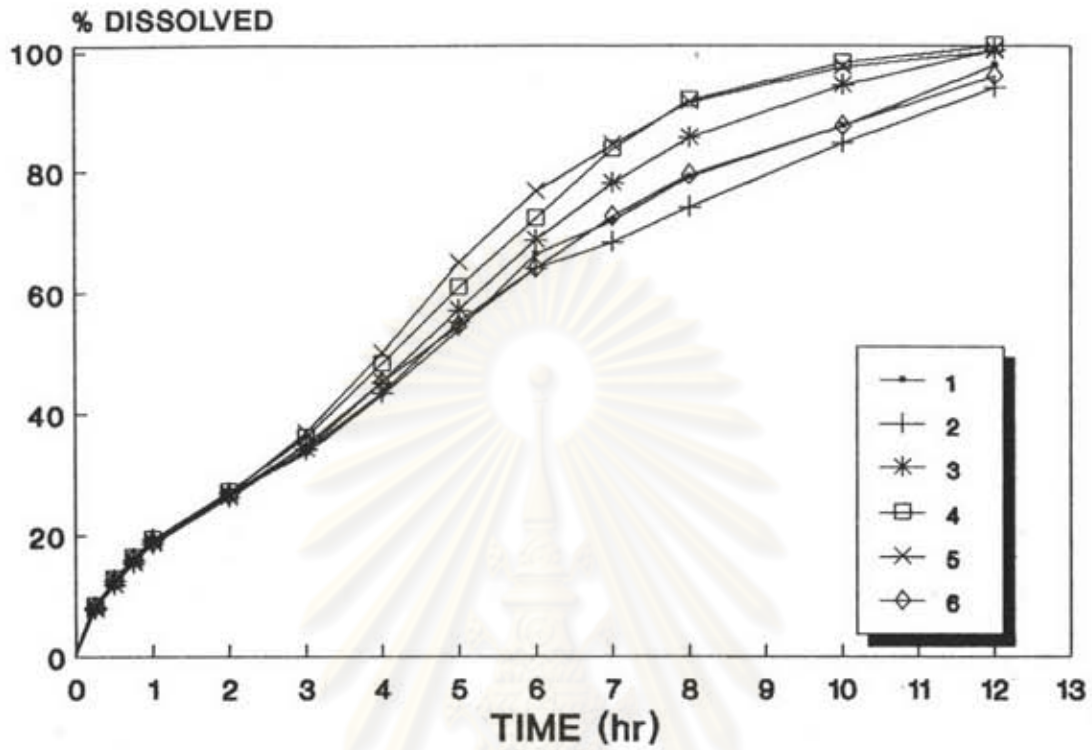

Figure 12 Dissolution Profiles of Six Tablets of Nuelin® Tested by pH Change Method

performed by Vipaluk (1993). The variation of drug release from product D seemed to be less than that from product E. However, this is only a little difference when compared to Theo-Dur® or Nuelin® either of which had the quite high value of standard deviation. A comparative plot of average amount of drug dissolved (Figure 13) indicated that all experimental tablets exhibited different patterns of drug release. In addition, the tablets prepared by fluidization coating appeared to have a slower release rate at the beginning which was similar to Theo-Dur®. The dissolution rate constants (Kd) reported in Table 11 were calculated from the slope of the first order plot between the amount of undissolved theophylline versus time in semi-logarithmic scale as the example demonstrated in Appendix C. The average of these constants decreased in the following order : product B > C > E > A > D. Statistical results, as presented in Table 12 and 13, exhibited no significant difference ($p > 0.05$) of dissolution rate constant among the formulations C, D, E and Theo-Dur®, however, Nuelin® had the fastest rate of all ($p < 0.05$).

2. In Vivo Evaluation

2.1 HPLC Analysis and Theophylline Concentrations in Plasma

Figure 14 shows chromatograms of deprotenized rabbit plasma, with and without theophylline, and an aqueous mixture of theophylline and β-hydroxyethyltheophylline (internal standard, IS) in mobile phase. Baseline resolution of theophylline from IS is achieved and the separation of them from endogenous substances are sufficient to avoid significant interference. Blank rabbit plasma also shows no peaks in the region of

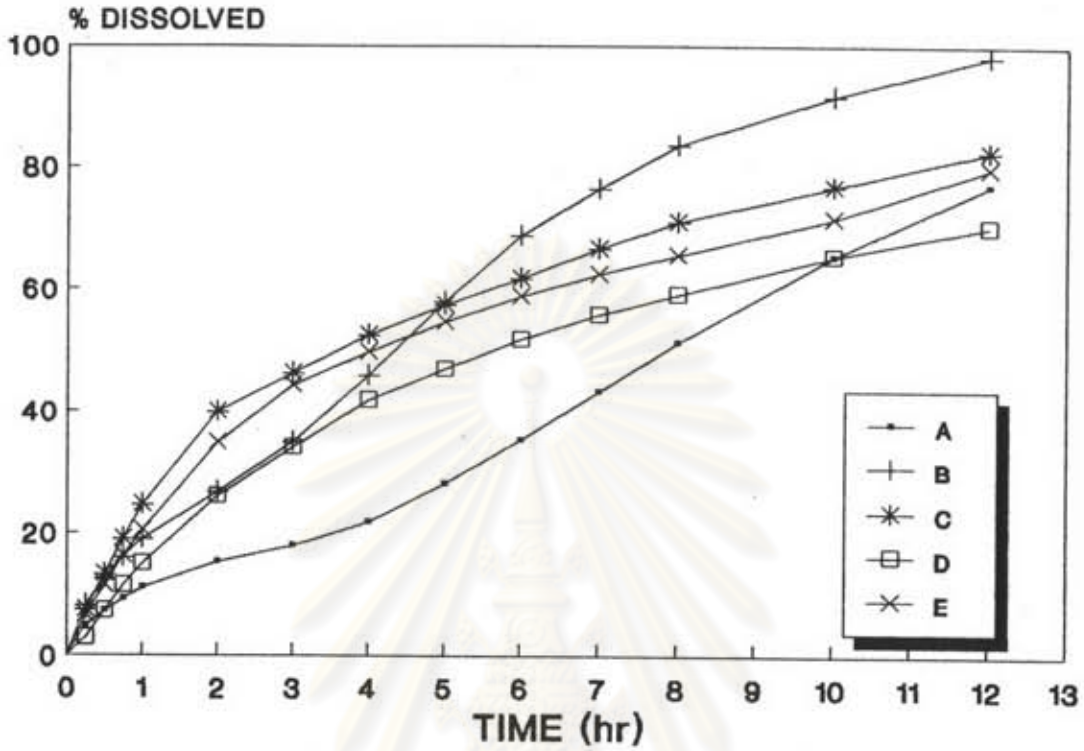

Figure 13 Dissolution Profiles of Five Formulations of Theophylline Sustained-release Tablets Tested by pH Change Method

Table 11 Dissolution Rate Constant (Kd) of Five Formulations of Theophylline Sustained-release Tablets

Tablet No.	Dissolution Rate Constant (hr^{-1})				
	A	B	C	D	E
1	0.046	0.113	0.059	0.044	0.056
2	0.053	0.090	0.060	0.040	0.046
3	0.056	0.113	0.059	0.044	0.060
4	0.045	0.151	0.061	0.037	0.065
5	0.033	0.145	0.060	0.049	0.047
6	0.054	0.102	0.057	0.052	0.050
$\overline{X}$	0.048	0.119	0.059	0.044	0.054
S.D.	0.003	0.010	0.001	0.002	0.003

Table 12 ANOVA for Dissolution Rate Constant (Kd) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	0.0227	0.0057	28.50
Within Group	25	0.0037	0.0002	
Total	29	0.0264		

$F^{e}_{0.05}(4, 25) = 2.76$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 13 Comparison of Dissolution Rate Constant between Formulations By Duncan's New Multiple Range Test

Formulations	Statistcal Significance
B and D	S
B and A	S
B and E	S
B and C	S
C and D	NS
C and A	NS
C and E	NS
E and D	NS
E and A	NS
A and D	NS

S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

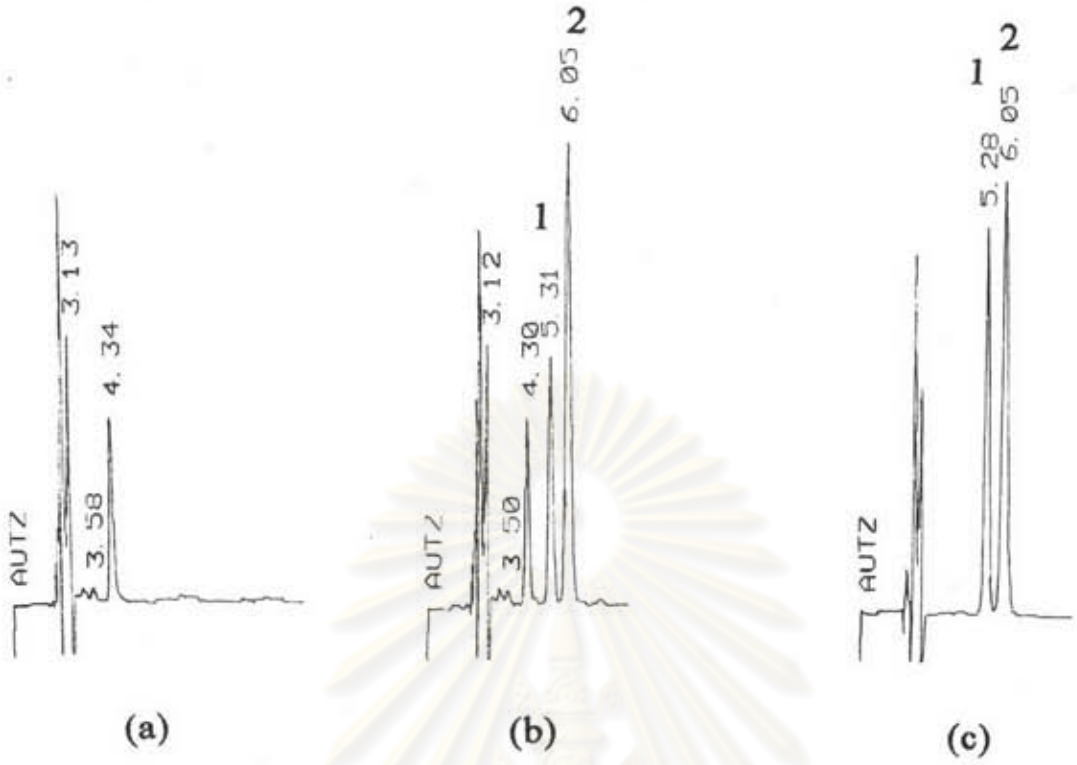

Figure 14 High Performance Liquid Chromatograms of :
(a) Blank Plasma (b) Spiked Plasma and Internal Standard (IS) and (c) Aqueous Mixture of Theophylline and IS in Mobile Phase

1 = Theophylline, 40 mcg/mL

2 = IS, 25 mcg/mL

theophylline or IS. The entire chromatogram was generated in less than 7 minutes.

Plasma theophylline concentrations at any sampling time interval up to 72 hours after oral administration of each product are presented in Tables 14 to 18 and Figures 15 to 19, respectively. The profiles revealed that theophylline concentrations were highly fluctuated among rabbits given the same formulation. In addition, the comparative plot of mean plasma concentrations also manifested the difference among formulations as shown in Figure 20.

2.2 Pharmacokinetic Parameters and Statistical Results

The relevant pharmacokinetic parameters derived from plasma concentration-time data are observed as follows.

2.2.1 Area under the Plasma Concentration-Time Curve (AUC_0^∞): The total amount of drug absorbed into the systemic circulation is represented by this parameter. The mean AUC_0^∞ for products A, B, C, D and E were 2.09 ± 0.25, 2.16 ± 0.17, 2.05 ± 0.19, 2.02 ± 0.17 and 2.04 ± 0.16 mg-hr/mL as the above order (Table 19). There were no statistically significant differences ($p > 0.05$) among AUC_0^∞ values of all formulations (Table 20).

This result obviously demonstrated that the amounts of theophylline absorbed were the same for tablets of all formulations.

Table 14 Plasma Theophylline Concentration (mcg/mL) from Eight Subjects after Oral Administration of Product A (Theo-Dur®)

Subject	Time (hr)									
No.	1.00	2.00	3.00	5.00	7.00	9.00	12.00	24.00	36.00	72.00
1	1.42	11.12	36.60	57.13	62.24	62.32	38.03	13.60	4.27	***
2	35.97	51.43	57.37	84.43	85.90	85.97	73.45	42.20	14.43	1.29
3	20.12	40.97	67.70	79.48	73.73	65.65	39.62	17.49	3.87	***
4	55.52	68.41	66.79	75.45	82.07	87.42	33.27	11.86	5.45	***
5	69.25	70.03	70.73	72.21	72.70	69.32	31.16	11.51	4.96	***
6	67.63	70.80	71.23	76.72	91.86	79.46	60.24	55.31	32.49	3.41
7	24.82	34.25	65.59	66.65	66.16	57.84	27.35	8.90	2.78	***
8	31.44	39.11	54.25	61.65	58.20	56.65	32.07	15.80	5.59	***
MEAN	38.27	48.27	61.28	71.72	74.11	70.58	41.90	22.08	9.23	0.59
SE	7.94	6.97	3.86	3.06	3.91	4.05	5.38	5.63	3.33	0.41

*** cannot be determined

Table 15 Plasma Theophylline Concentration (mcg/mL) from Eight Subjects after Oral Administration of Product B (Nuelin®)

Subject	Time (hr)									
No.	1.00	2.00	3.00	5.00	7.00	9.00	12.00	24.00	36.00	72.00
1	34.89	45.89	55.75	65.04	66.11	70.75	45.39	13.54	4.18	***
2	40.66	60.59	72.13	81.22	77.65	81.29	54.43	29.96	4.92	***
3	58.83	62.84	65.52	66.93	75.38	61.79	36.08	15.94	5.17	***
4	46.16	57.07	63.48	63.62	64.40	61.16	46.23	23.20	13.55	2.56
5	22.14	30.59	50.45	52.56	48.69	45.31	33.97	24.75	6.16	***
6	15.38	20.73	43.76	46.44	42.21	32.92	19.96	12.42	7.99	***
7	26.86	36.86	42.92	49.04	48.90	47.78	36.51	22.78	15.24	2.92
8	35.38	39.82	47.56	48.90	52.42	50.52	33.06	13.97	4.04	***
MEAN	35.04	44.30	55.20	59.22	59.47	56.44	38.20	19.57	7.66	0.69
SE	4.58	4.99	3.59	3.98	4.38	5.09	3.45	2.13	1.44	0.42

*** cannot be determined

Table 16 Plasma Theophylline Concentration (mcg/mL) from Eight Subjects after Oral Administration of Product C

Subject	Time (hr)									
No.	1.00	2.00	3.00	5.00	7.00	9.00	12.00	24.00	36.00	72.00
1	7.67	14.42	33.89	40.86	50.93	72.82	61.84	30.25	12.22	5.17
2	27.44	51.82	68.90	71.67	68.46	68.02	65.83	30.43	14.08	1.74
3	74.16	76.77	77.25	80.67	82.18	80.67	46.97	23.75	6.49	***
4	41.64	56.74	77.65	81.15	81.57	79.75	61.50	22.27	6.25	1.01
5	16.79	25.03	46.08	52.28	66.79	55.59	26.08	4.96	2.00	***
6	32.14	58.02	74.40	82.51	70.29	66.03	65.14	28.59	7.02	***
7	12.07	24.82	42.56	54.22	68.81	59.32	23.07	4.26	1.14	***
8	50.03	55.52	65.24	70.73	62.99	61.37	30.73	14.82	4.32	***
MEAN	32.74	45.39	60.75	66.76	69.00	67.95	47.65	19.92	6.69	0.99
SE	7.32	7.09	5.73	5.19	3.31	3.06	6.11	3.55	1.50	0.60

*** cannot be determined

Table 17 Plasma Theophylline Concentration (mcg/mL) from Eight Subjects after Oral Administration of Product D

Subject	Time (hr)									
No.	1.00	2.00	3.00	5.00	7.00	9.00	12.00	24.00	36.00	72.00
1	29.45	31.26	40.64	47.35	55.46	56.86	30.92	9.52	5.67	***
2	10.69	15.03	23.63	60.40	69.37	75.32	56.06	29.44	12.16	2.60
3	8.49	13.24	37.02	44.85	58.28	63.31	42.27	25.76	8.49	***
4	18.69	49.04	59.18	66.23	74.14	80.02	72.36	33.27	19.75	2.63
5	2.84	9.61	56.16	59.46	60.94	57.14	42.63	17.56	4.61	***
6	18.48	37.78	40.38	44.61	47.99	53.20	32.49	14.89	6.86	***
7	52.14	52.35	61.23	64.40	74.11	70.31	59.40	24.75	7.28	***
8	41.08	42.42	53.62	64.11	64.82	57.21	45.17	28.90	17.92	4.18
MEAN	22.73	31.34	46.48	56.43	63.14	64.17	47.66	23.01	10.34	1.18
SE	5.62	5.57	4.34	3.06	3.06	3.27	4.66	2.71	1.89	0.56

*** cannot be determined

Table 18 Plasma Theophylline Concentration (mcg/mL) from Eight Subjects after Oral Administration of Product E

Subject	Time (hr)									
No.	1.00	2.00	3.00	5.00	7.00	9.00	12.00	24.00	36.00	72.00
1	17.97	38.45	59.08	78.31	69.08	64.47	31.39	5.87	3.84	***
2	19.50	60.48	64.33	67.06	81.74	85.94	51.67	10.76	2.79	***
3	36.29	49.85	86.01	88.75	90.74	93.00	51.77	13.89	3.14	***
4	48.19	57.70	72.39	86.93	93.50	84.06	72.74	31.90	14.13	1.97
5	59.89	71.42	83.60	81.55	81.04	67.12	28.35	6.37	1.86	***
6	54.41	64.18	76.08	81.86	79.75	69.11	32.21	8.48	2.28	***
7	40.59	79.04	79.82	80.10	80.17	76.79	65.31	6.44	2.84	***
8	42.78	49.32	55.38	59.04	53.48	47.00	36.72	14.89	5.94	1.79
MEAN	39.95	58.80	72.09	77.95	78.69	73.44	46.27	12.32	4.60	0.47
SE	5.00	4.30	3.77	3.32	4.17	4.84	5.53	2.85	1.34	0.29

*** cannot be determined

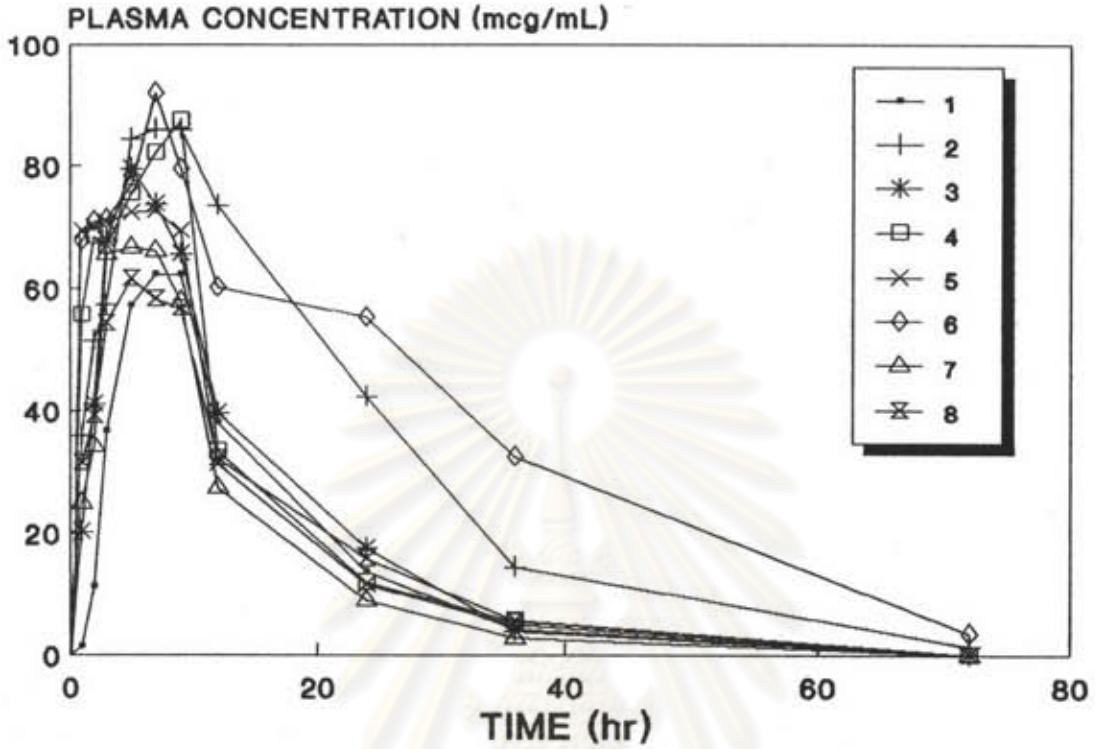

Figure 15 Plasma Theophylline Concentration-Time Profiles from Eight Subjects After Oral Administration of Product A

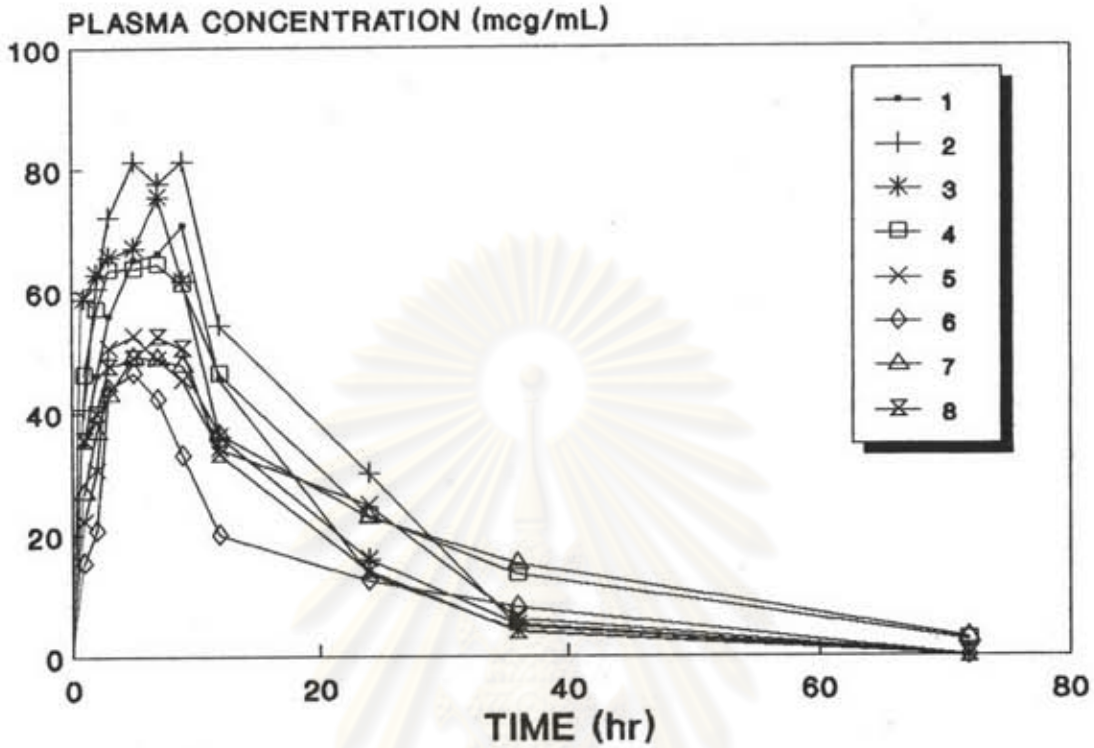

Figure 16 Plasma Theophylline Concentration-Time Profiles from Eight Subjects After Oral Administration of Product B

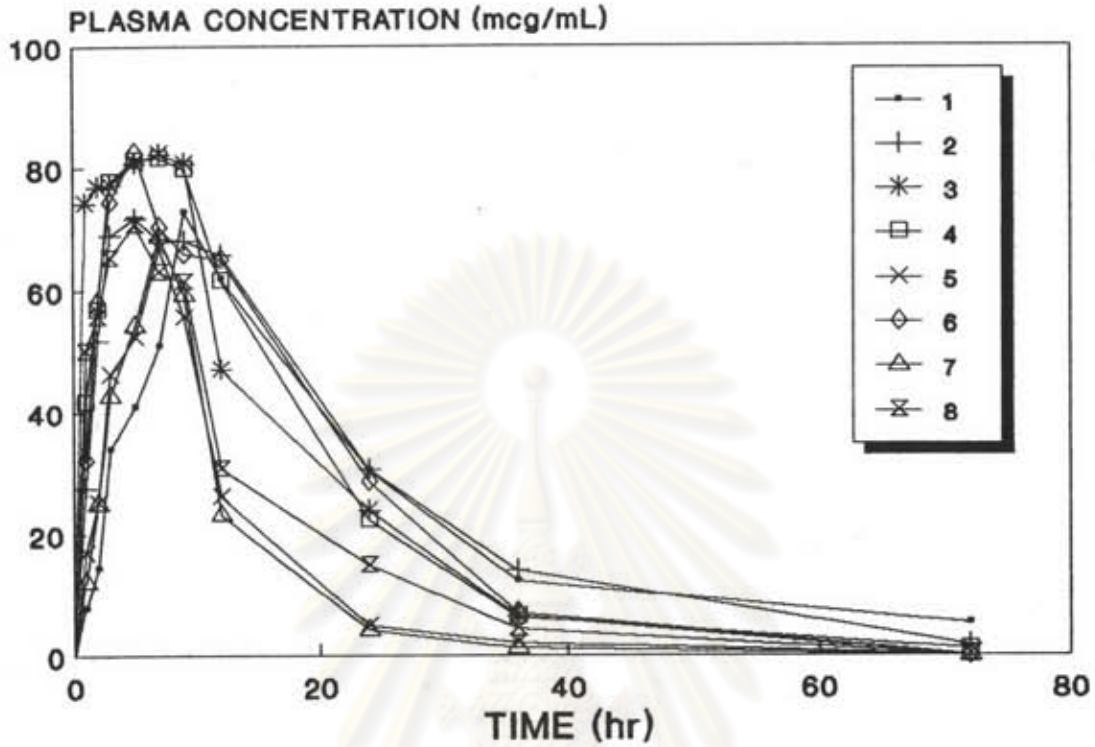

Figure 17 Plasma Theophylline Concentration-Time Profiles from Eight Subjects After Oral Administration of Product C

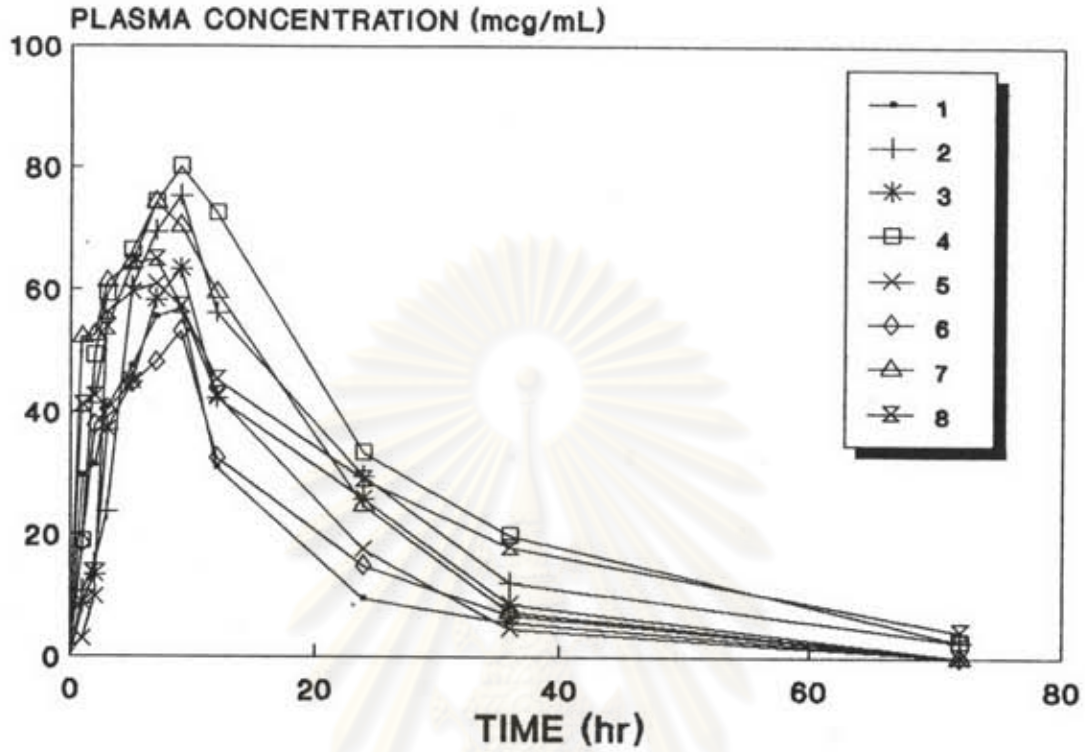

Figure 18 Plasma Theophylline Concentration-Time profiles from Eight Subjects After Oral Administration of Product D

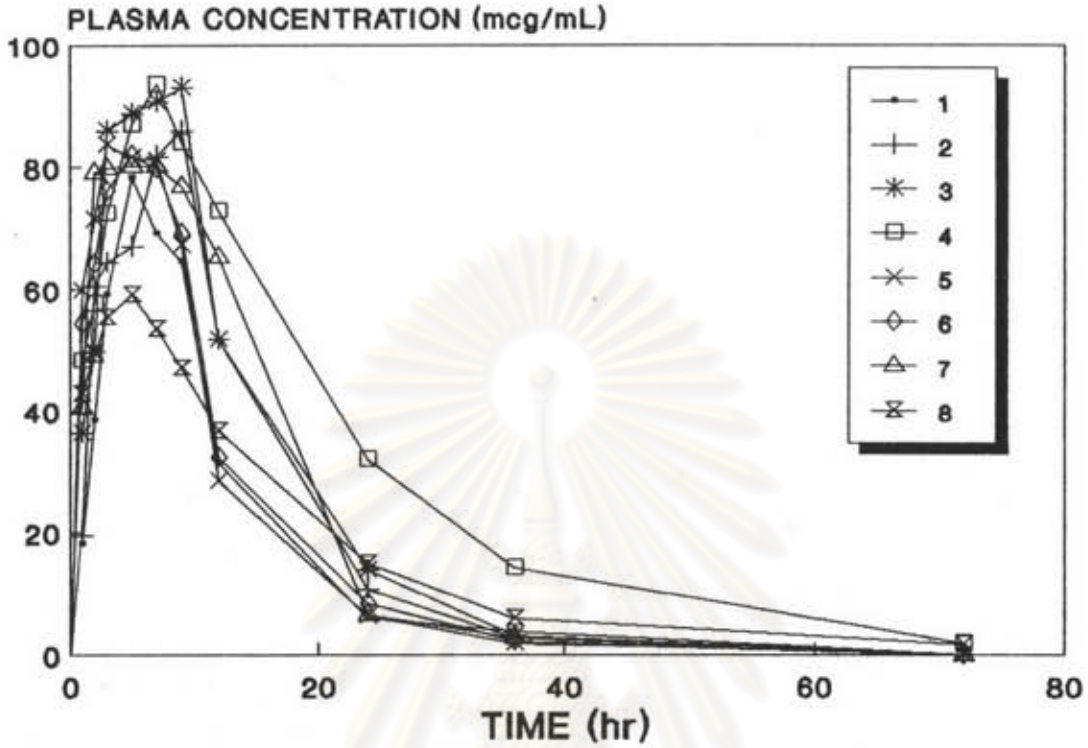

Figure 19 Plasma Theophylline Concentration-Time profiles from Eight Subjects After Oral Administration of Product E

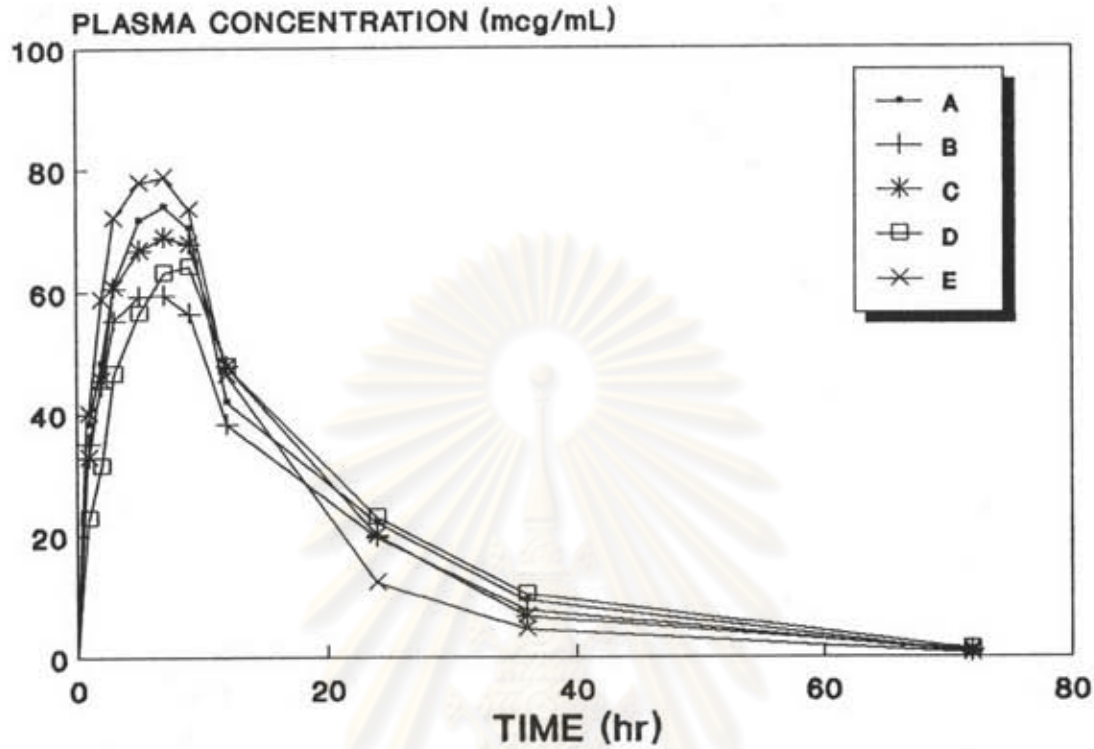

Figure 20 Mean Plasma Theophylline Concentration-Time Profiles of Five Formulations of Theophylline Sustained-release Tablets

Table 19 Area under the Plasma Concentration-Time Curve (AUC_0^∞) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	AUC_0^∞ (mg-hr/mL)				
	A	B*	C	D	E
1	1.55	2.24	2.36	1.44	1.57
2	1.86	2.89	1.28	1.81	2.94
3	2.01	2.22	2.33	1.67	2.08
4	1.83	1.91	2.58	1.50	1.78
5	1.61	1.37	1.24	2.29	2.20
6	1.47	1.78	1.71	2.27	1.73
7	2.99	2.64	2.46	2.87	2.30
8	3.42	2.21	2.42	2.29	1.65
$\bar{X}$	2.09	2.16	2.05	2.02	2.04
S.D.	0.25	0.17	0.19	0.17	0.16

* normalized to 300 mg

Table 20 ANOVA for Area under the Plasma Concentration-Time Curve (AUC_0^∞) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	0.0998	0.0250	0.083
Within Group	35	10.5080	0.3002	
Total	39	10.6078		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

In this study the mean relative bioavailability was also calculated by comparing the mean AUC_0^∞ of each product to that of Theo-Dur® which used as a reference. The values obtained for products B, C, D and E were 103.35 %, 98.09 %, 96.65 % and 97.61 % respectively.

2.2.2 Time to Peak Plasma Concentration (t_{max}) : This parameter is generally used to indicate the onset of drug action. The shorter the time to peak plasma concentration was, the faster the drug was absorbed. As presented in Table 21, the average times were 9.38 ± 0.82, 9.00 ± 0.73, 9.25 ± 0.67, 10.88 ± 0.55 and 8.50 ± 0.89 hours for products A, B, C, D and E respectively. One way analysis of variance (Table 22) showed no statistically significant differences ($p > 0.05$) among t_{max} values of all formulations.

This suggested that every sustained-release theophylline tablet provided the same onset of action although their individual release mechanisms were quite different.

2.2.3 Peak Plasma Concentration (C_{max}) : This is a parameter which was employed to indicate the intensity of action of a drug product. The mean peak plasma concentrations for products A, B, C, D and E were 76.01 ± 4.19, 73.84 ± 5.61, 68.26 ± 4.69, 68.19 ± 4.91 and 82.11 ± 3.88 mcg/mL respectively (Table 23). There were no statistically significant differences ($p > 0.05$) among C_{max} values of the five formulations tested (Table 24).

Table 21 Time to Peak Plasma Concentration (t_{max}) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	t_{max} (hr)				
	A	B	C	D	E
1	12.00	12.00	12.00	12.00	9.00
2	12.00	12.00	7.00	12.00	7.00
3	7.00	9.00	9.00	12.00	12.00
4	12.00	9.00	9.00	12.00	12.00
5	9.00	7.00	9.00	9.00	5.00
6	7.00	7.00	7.00	12.00	7.00
7	9.00	7.00	9.00	9.00	9.00
8	7.00	9.00	12.00	9.00	7.00
$\overline{X}$	9.38	9.00	9.25	10.88	8.50
S.D.	0.82	0.73	0.67	0.55	0.89

Table 22 ANOVA for Time to Peak Plasma Concentration (t_{max}) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	25.35	6.34	1.44
Within Group	35	154.25	4.41	
Total	39	179.60		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 23 Peak Plasma Concentration(C_{max}) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	C_{max} (mcg/mL)				
	A	B	C	D	E
1	62.32	84.90	72.82	56.86	93.50
2	85.97	97.56	71.67	75.32	78.31
3	79.48	90.46	82.18	63.31	85.94
4	87.42	77.28	81.57	80.02	93.00
5	72.70	63.07	66.79	60.94	79.75
6	61.65	55.73	82.51	53.20	80.17
7	91.86	58.85	68.81	74.11	59.04
8	66.65	62.90	70.73	64.82	87.20
$\overline{X}$	76.01	73.84	68.26	68.19	82.11
S.D.	4.19	5.61	4.69	4.91	3.88

Table 24 ANOVA for Peak Plasma Concentration (C_{max}) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	1053.64	263.41	2.04
Within Group	35	4511.25	128.89	
Total	39	0.0264		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Because there were no statistically significant differences in AUC_0^∞, t_{max} and C_{max} among all five formulations tested, it could be stated that the experimental tablets prepared by different techniques were bioequivalent to Theo-Dur® and Nuelin® in terms of the rate and the extent of absorption.

From the plasma theophylline concentration-time curve, it could be stated that the pharmacokinetics of theophylline in rabbits after oral drug administration could be explained by a two compartment model with first-order absorption and elimination.

2.2.4 Absorption Rate Constant (K_a) : The average absorption rate constants for products A, B, C, D and E were 0.16 ± 0.04, 0.20 ± 0.18, 0.15 ± 0.04, 0.15 ± 0.03 and 0.17 ± 0.04 hr^{-1} respectively (Table 25). Statistical analysis resulted in Table 26 indicated that there were no significant differences ($p > 0.05$) among K_a values of all formulations. That is to say all formulations were absorbed at the same rate.

It should be noted that the absorption rate was independent of the rate of drug dissolution. For instance, even though Nuelin® SR showed higher dissolution rate than the others (Table 11), the absorption rate of all formulations were still the same. This result was consistent with the experiment which used t_{max} as a parameter to compare the rate of drug absorption.

Table 25 Absorption Rate Constant (K_a) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	K_a (hr^{-1})				
	A	B	C	D	E
1	0.16	0.15	0.12	0.20	0.26
2	0.12	0.13	0.11	0.14	0.16
3	0.15	0.14	0.14	0.15	0.16
4	0.21	0.12	0.13	0.12	0.13
5	0.21	0.12	0.22	0.14	0.21
6	0.10	0.64	0.12	0.17	0.17
7	0.19	0.17	0.21	0.12	0.16
8	0.16	0.14	0.14	0.17	0.12
$\bar{X}$	0.16	0.20	0.15	0.15	0.17
S.D.	0.04	0.18	0.04	0.03	0.04

Table 26 ANOVA for Absorption Rate Constant (K_a) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	0.0147	0.0037	0.50
Within Group	35	0.2590	0.0074	
Total	39	0.2737		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

2.2.5 Elimination Rate Constant (K_{el}) : Table 27 shows elimination rate constants of each plasma concentration-time profile including the average values which were 0.08±0.01, 0.07±0.02, 0.09±0.02, 0.07±0.02 and 0.10±0.02 hr^{-1} for products A, B, C, D and E respectively. These values decreased in the following order : E > C > A > B = D. The elimination rate constant of product E was significantly more ($p > 0.05$) than that of product B and D, as presented in Tables 28 and 29.

This was probably owing to the variation of drug excretion among the tested rabbits because the absorption rate of all drug products were found to be the same.

2.2.6 Biological Half-life ($t_{1/2}$) : The mean half-lives of theophylline for products A, B, C, D and E were 8.62±1.38, 11.01±4.72, 8.36±1.65, 10.70±3.13 and 7.18±1.85 hours as the above order (Table 30). Statistical analysis showed no significant differences among half-life values of all formulations (Table 31).

The average of pharmacokinetic parameters of theophylline in rabbits following oral ingestion of five sustained-release theophylline tablets were summarized in Table 32. Statistical analysis of these corresponding parameters, except K_{el}, demonstrated that the three experimental tablets (C, D, E) were bioequivalent to each other and also to commercial tablets, i.e. Theo-Dur® and Nuelin® .

Table 27 Elimination Rate Constant (K_{el}) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	K_{el} (hr^{-1})				
	A	B	C	D	E
1	0.09	0.10	0.07	0.07	0.08
2	0.09	0.10	0.06	0.06	0.12
3	0.10	0.08	0.08	0.07	0.12
4	0.08	0.06	0.08	0.07	0.07
5	0.07	0.07	0.09	0.09	0.11
6	0.06	0.04	0.09	0.06	0.11
7	0.10	0.04	0.12	0.09	0.13
8	0.07	0.09	0.08	0.04	0.07
$\overline{X}$	0.08	0.07	0.09	0.07	0.10
S.D.	0.01	0.02	0.02	0.02	0.02

Table 28 ANOVA for Elimination Rate Constant (K_{el}) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	0.0054	0.00135	3.46
Within Group	35	0.0136	0.00039	
Total	39	0.0190		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 29 Comparison of Elimination Rate Constant (K_{el}) between Formulations by Duncan's New Multiple Range Test

Formulations	Statistcal Significance
E and D	S
E and B	S
E and A	NS
E and C	NS
C and D	NS
C and B	NS
C and A	NS
A and D	NS
A and B	NS
B and D	NS

S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

Table 30 Biological Half-life ($t_{1/2}$) of Theophylline Following Oral Administration of Five Formulations of Theophylline Sustained-release Tablets

Subject No.	$t_{1/2}$ (hr)				
	A	B	C	D	E
1	7.62	7.00	10.34	10.34	8.88
2	8.06	6.86	10.83	10.83	5.68
3	7.14	8.55	8.35	10.34	6.03
4	8.88	11.55	8.15	10.04	10.04
5	9.36	9.76	7.53	7.53	6.13
6	11.18	17.77	7.53	10.83	6.30
7	7.22	18.73	5.59	7.88	5.29
8	9.49	7.88	8.56	17.77	9.12
$\bar{X}$	8.62	11.01	8.36	10.70	7.18
S.D.	1.38	4.72	1.65	3.13	1.85

Table 31 ANOVA for Biological Half-life ($t_{1/2}$) of Five Formulations of Theophylline Sustained-release Tablets

Source of Variation	d.f. [a]	SS [b]	MS [c]	F-value [d]
Among Group	4	85.0042	21.2510	2.64
Within Group	35	281.4162	8.0405	
Total	39	366.4204		

$F^{e}_{0.05}(4, 30) = 2.69$

a = Degree of Freedom

b = Sum of Square

c = Mean Square

d = Variance Ratio

e = F-value obtained from the table

Table 32 Estimated Pharmacokinetic Parameters (Mean (S.D.)) of Theophylline from 8 Subjects Following Oral Administration of Theophylline Sustained-release Tablets

Parameters	Formulations					F-test	Statistical Significant at 95% Confident interval
	A	B	C	D	E		
C_{max}	76.01(4.19)	73.84(5.61)	68.26(4.69)	68.19(4.91)	82.11(3.88)	2.04	NS
t_{max}	9.38(0.82)	9.00(0.73)	9.25(0.67)	10.88(0.55)	8.50(0.89)	1.44	NS
AUC_0^∞	2.09(0.25)	2.16(0.17)	2.05(0.19)	2.02(0.17)	2.04(0.16)	0.083	NS
$t_{1/2}$	8.62(1.38)	11.01(4.72)	8.36(1.65)	10.70(3.13)	7.18(1.85)	2.64	NS
K_a	0.16(0.04)	0.20(0.18)	0.15(0.04)	0.15(0.03)	0.17(0.04)	0.50	NS
K_{el}	0.08(0.01)	0.07(0.02)	0.09(0.02)	0.07(0.02)	0.10(0.02)	3.46	S*

* K_{el} of Product E was more than that of product D and B

2.3 In Vitro - In Vivo Correlation

As presented in Table 33, It was AUC_0^∞, not C_{max} or t_{max}, that showed statistically significant correlation with the dissolution rate constant ($p > 0.05$). This was due to the fact that both Kd and AUC_0^∞ were calculated using total data from dissolution profile and plasma concentration-time curve respectively. In contrast, C_{max} and t_{max} were obtained from only at a specific time which the absorption was yet incompleted.

From this correlation, it is particularly useful to employ the suitable Kd as an important parameter to control the lot-to-lot variation in the manufacturing process instead of repeating the bioavailability test for each time of production to assure the product's efficacy.

Table 33 In Vitro-In Vivo Correlations

Correlation (n=5)	Correlation Coefficient	t value	Statistical Significance
Kd vs C_{max}	0.03	0.059	NS
Kd vs t_{max}	-0.38	0.707	NS
Kd vs AUC_0^∞	0.88	3.225	S

$$t_{(0.05, 3)}^{a} = 3.182$$

a = t value obtained from the table

S = significant correlation at $p < 0.05$

NS = no significant correlation at $p > 0.05$