CHAPTER III
RESULTS AND DISCUSSION

A. *In vitro study*

1. Formulation of stavudine pellet

The formulations of stavudine pellets with various ratios of active ingredient, fillers and water amount are presented in Table 9 The range of water amount used in each formulation was optimized to provide the consistent pellets and the water amount giving the best characteristic pellets regarding the shape and size distribution, was chosen to represent the optimum water of each formulation. The results showed that the amount of water required was different for the different formulations. The range of water amount used to perform the consistent pellets in each formulation was very narrow. It was observed that the more Avicel® PH101 used in formulation, the more quantity of water was required due to Avicel® PH101 has high porosity and by natural it can be absorbed water very much to give good plastic mass property for extrudates. Increasing high solubility ingredients (lactose or stavudine) in formulation lead to using the less quantity of water indicating the solubility of materials used (both drugs and fillers) played an important role in the quantity of water required to form satisfactory pellets. In this study, the optimum water amount of each formulation were approximately 55 %w/w to 65 %w/w based on dry basis.

Table 9 The range of water amount used in each formulation

Ingredient (%w/w)	P1	P2	P3	P4	P5
Stavudine	40	40	40	40	60
Avicel® PH101	40	30	20	60	40
Lactose	20	30	40	-	-
Range of water amount	60-62	58-60	55-57	60-65	60-63
Optimum water amount (base on dry basis)	62	58	55	65	60

2. **Appearance and Sphericity of pellets**

The microscopic appearance of stavudine pellet formulation P1 are presented in Figure 9. At 400 rpm of spheronizer speed, dog bone pellets were obtained. When the spheronizer speed and spheronization time increased, the shape of pellets was more spherical with smoother surface.

The microscopic appearance of stavudine pellet formulation P2 are presented in Figure 10. At 400 rpm of spheronizer speed, short rod shape pellets combined with dog bone pellets were obtained. When the spheronizer speed and spheronization time increased, shorter rod shape pellets with smoother surface were obtained. At 860 rpm of spheronizer speed and 15 minutes of spheronization time, the shape of pellets was more spherical with smoother surface.

The microscopic appearance of stavudine pellet formulation P3 are presented in Figure 11. At 400 rpm of spheronizer speed, rod shape pellets were obtained. When the spheronizer speed and spheronization time increased, shorter rod shape pellets with smoother surface were obtained. However, at 860 rpm of spheronizer speed and 15 minutes of spheronization time, the shape of pellets still was short rod but the surface was smoother.

The microscopic appearance of stavudine pellet formulation P4 and P5 are presented in Figure 12 to 13. At 400 rpm of spheronizer speed, dog bone combined with spherical pellets were obtained. When the spheronizer speed and spheronization time increased, sphere shape pellets with smoother surface were obtained. At 860 rpm of spheronizer speed and 15 minutes of spheronization time, the shape of pellets was quite sphere with smoothest surface.

The parameter for sphericity measurement of stavudine pellet is shown in term of aspect ratio in Table 10. The results indicated that when the spheronizer speed and spheronization time increased then aspect ratio decreased. In each formulation, at 400 rpm of spheronizer speed and 5 minutes of spheronization time the aspect ratio was higher than the other condition whereas at 860 rpm of spheronizer speed and 15 minutes of spheronization time the aspect ratio was lowest and it closed to 1. At the same spheronizer speed and spheronization time, stavudine pellet formulation P1, P2 and P3 which used lactose as a diluent had aspect ratio higher than formulation P4 and P5 which used Avicel® PH101 as a diluent.

These results complied with the microscopic appearance which showed that the shape of pellets was more spherical when the spheronizer speed and spheronization time increased. Besides, pellets using Avicel® PH101 in the formulation were more spherical than pellets using lactose as a diluent.

These results obviously indicated that spheronizer speed and spheronization time had important impact on the appearance and sphericity of stavudine pellet. Forming the suitably shaped pellets during spheronization step will require the extrudates with sufficiently plastic property that are spheronized by the force which occurred from movement of the friction plate of the spheronizer. With friction, the extrudates are initially broken down into a short length and later form spherical pellets. Therefore, in all spheronization times, pellets obtained from higher spheronizer speed were more spherical than those from low spheronizer speed. This result corresponsed with Kaisri et al. (1999) who explain that this may be due to the forces from higher speeds were more than those from low spheronizer speed, increasing spheronization time means increases the time to apply the forces. Therefore, increasing shorter rod shape or increasing sphericity with smooth surface of stavudine pellet were obtained from low or high spheronizer speed, respectively.

In formulation P1, P2 and P3 which use lactose as a filler, when spheronization time was increased, a shorter rod shape were obtained at low spheronizer speeds and a sphericity with a smooth surface was obtain at high spheronizer speeds. However, the lumps of pellets were found when spheronization time was increased as well. For formulation P4 and P5 which used Avicel® PH101 as a filler and no lactose in formulation, pellets were more sphere either at low spheronizer speeds or low spheronization time. The lumps of pellets were not found when spheronization time was increased. These results indicated that the usage of microcrystalline cellulose as a filler in the formulation can form the satisfied shape of stavudine pellet more than pellets using lactose as a filler. When amount of Avicel® PH101 was increased, the more spherical pellets were obtained as shown that stavudine pellet in formulation P4 were more spherical than formulation P5. This can be explained that Avicel® PH101 has a spheronization enhancer property because of its high internal porosity and large surface area, it provides highly absorbent and moisture retaining properties which are essential to the extrusion process. The retained moisture helps to manipulate the shape of pellet during spheronization. In addition, because the properties of both stavudine and lactose are having high solubility and binding property, therefore it could be possible that if stavudine and lactose were used together in the formulation, extrudates would be very sticky and caused the lumps of pellets during spheronization step.

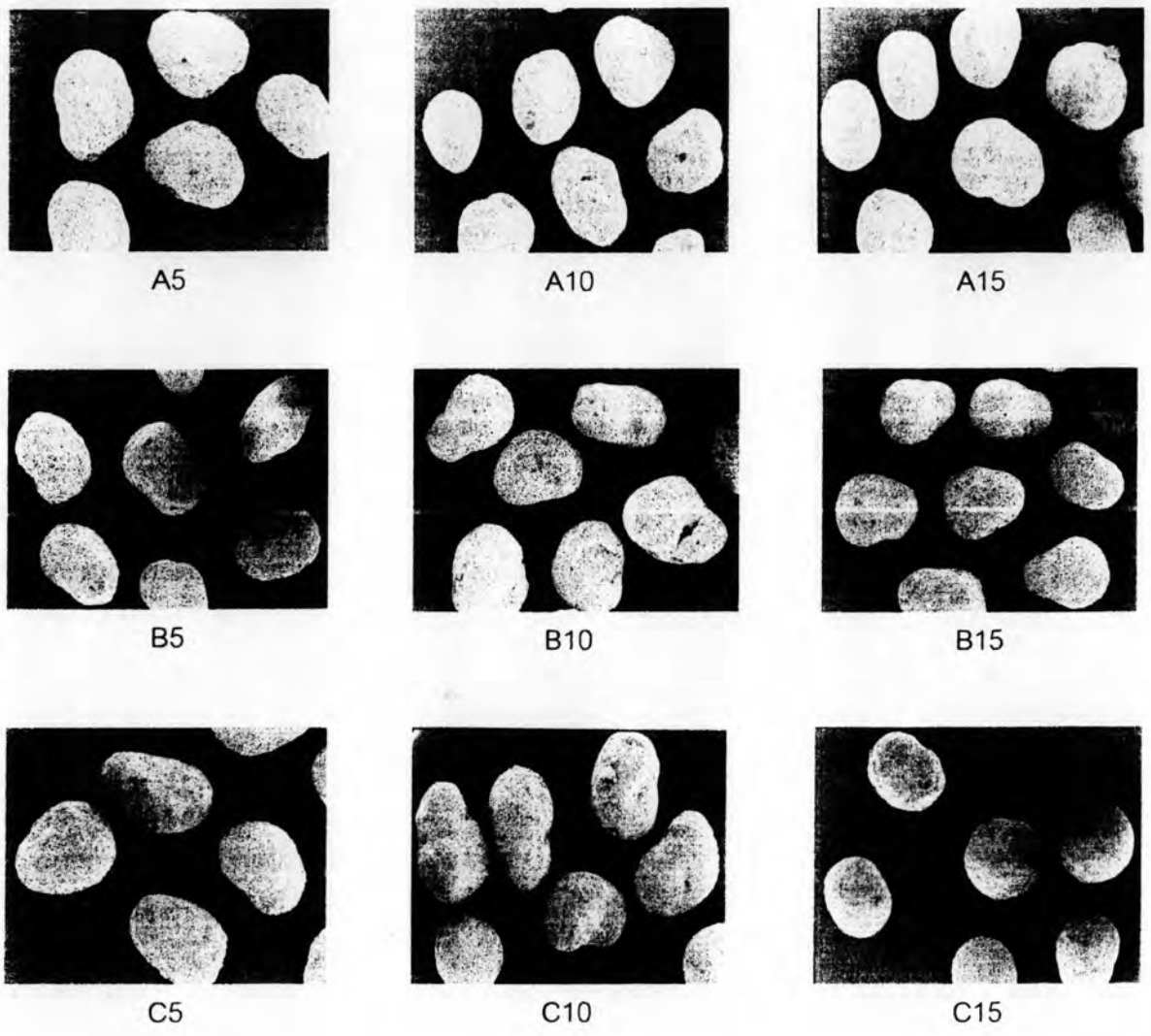


Figure 9 Photomicrographs of stavudine pellets (Formulation P1) prepared with various spheronization times and spheronizer speeds (×25)

(A5, A10, A15 are stavudine pellet used spheronizer speed of 400 rpm and spheronization times 5, 10, 15 mins ;

B5, B10, B15 are stavudine pellet used spheronizer speed of 600 rpm and spheronization times 5, 10, 15 mins ;

C5, C10, C15 are stavudine pellet used spheronizer speed of 860 rpm and spheronization times 5, 10, 15 mins)

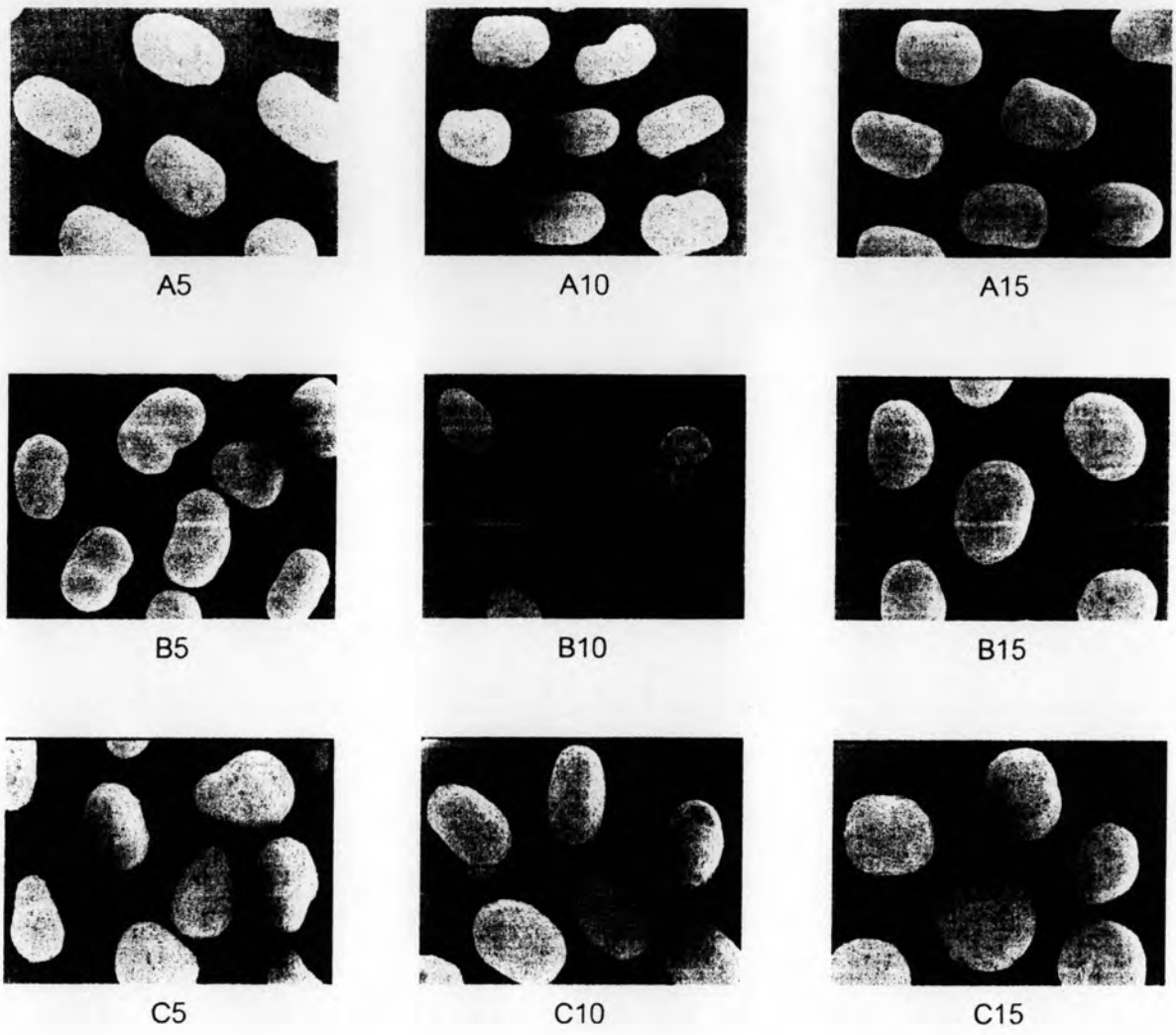


Figure 10 Photomicrographs of stavudine pellets (Formulation P2) prepared with various spheronization times and spheronizer speeds (×25)

(A5, A10, A15 are stavudine pellet used spheronizer speed of 400 rpm and spheronization times 5, 10, 15 mins ;

B5, B10, B15 are stavudine pellet used spheronizer speed of 600 rpm and spheronization times 5, 10, 15 mins ;

C5, C10, C15 are stavudine pellet used spheronizer speed of 860 rpm and spheronization times 5, 10, 15 mins)

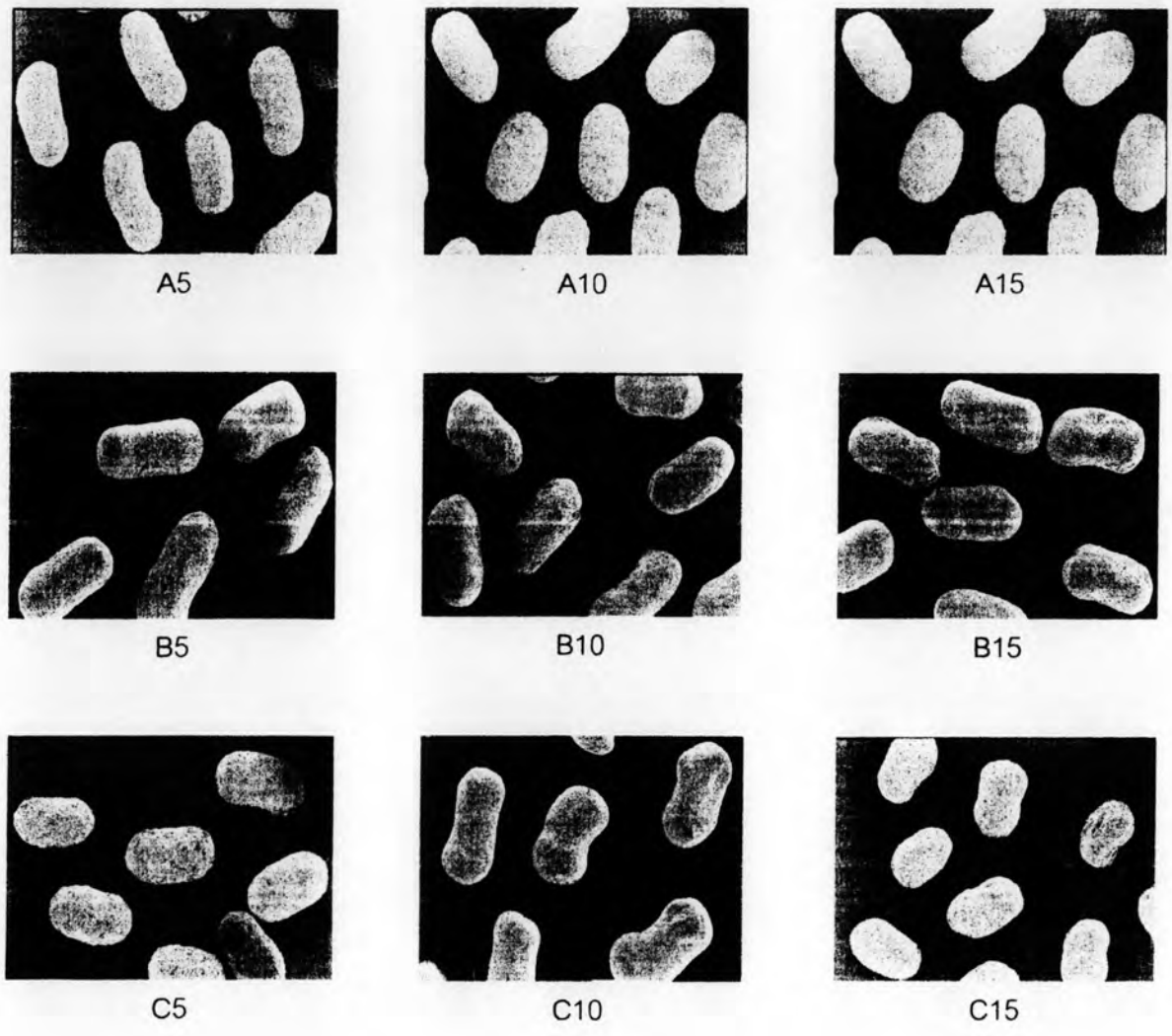


Figure 11 Photomicrographs of stavudine pellets (Formulation P3) prepared with various spheronization times and spheronizer speeds (×25)

(A5, A10, A15 are stavudine pellet used spheronizer speed of 400 rpm and spheronization times 5, 10, 15 mins ;

B5, B10, B15 are stavudine pellet used spheronizer speed of 600 rpm and spheronization times 5, 10, 15 mins ;

C5, C10, C15 are stavudine pellet used spheronizer speed of 860 rpm and spheronization times 5, 10, 15 mins)

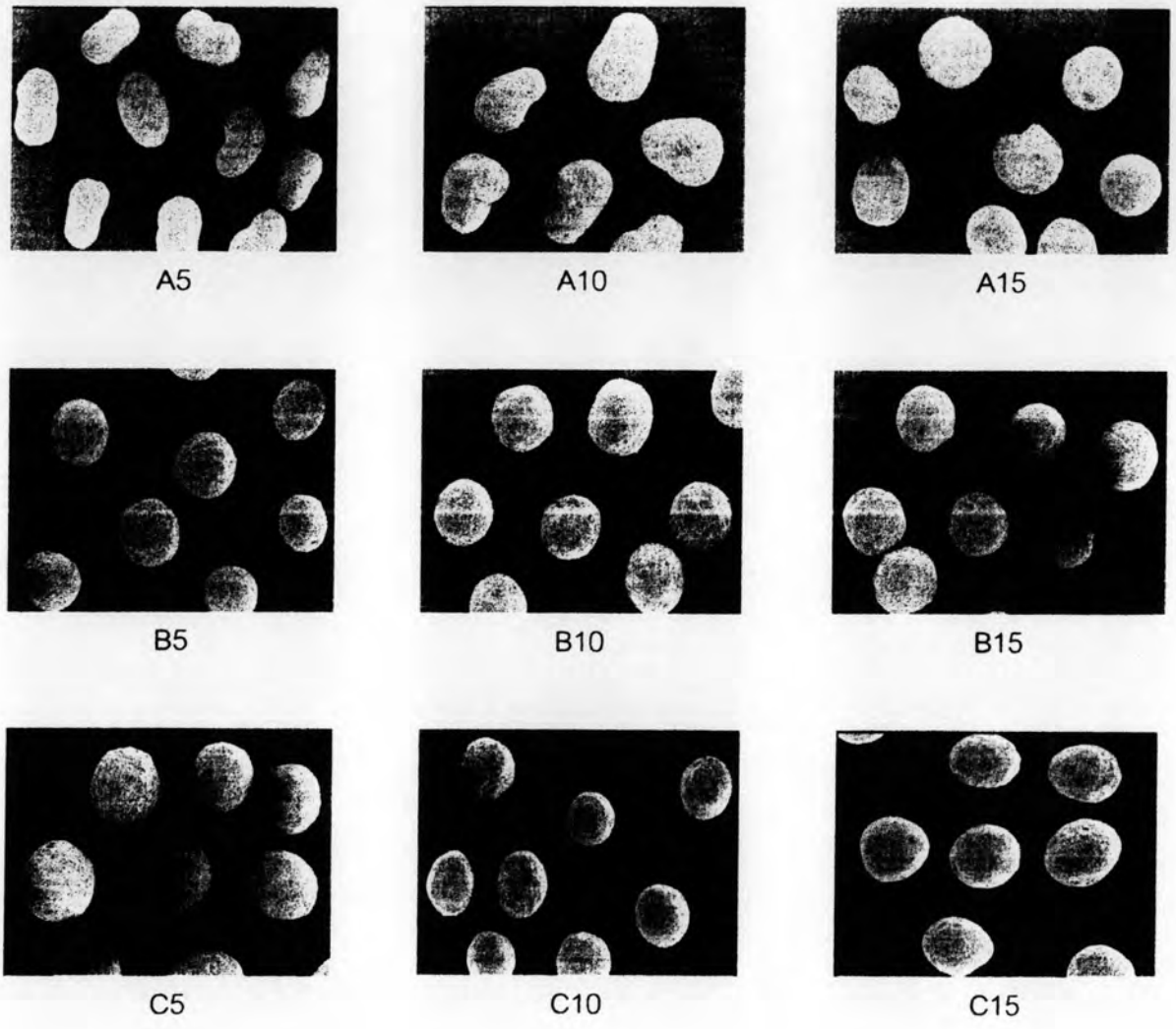


Figure 12 Photomicrographs of stavudine pellets (Formulation P4) prepared with various spheronization times and spheronizer speeds (X25)

(A5, A10, A15 are stavudine pellet used spheronizer speed of 400 rpm and spheronization times 5, 10, 15 mins ;

B5, B10, B15 are stavudine pellet used spheronizer speed of 600 rpm and spheronization times 5, 10, 15 mins ;

C5, C10, C15 are stavudine pellet used spheronizer speed of 860 rpm and spheronization times 5, 10, 15 mins)

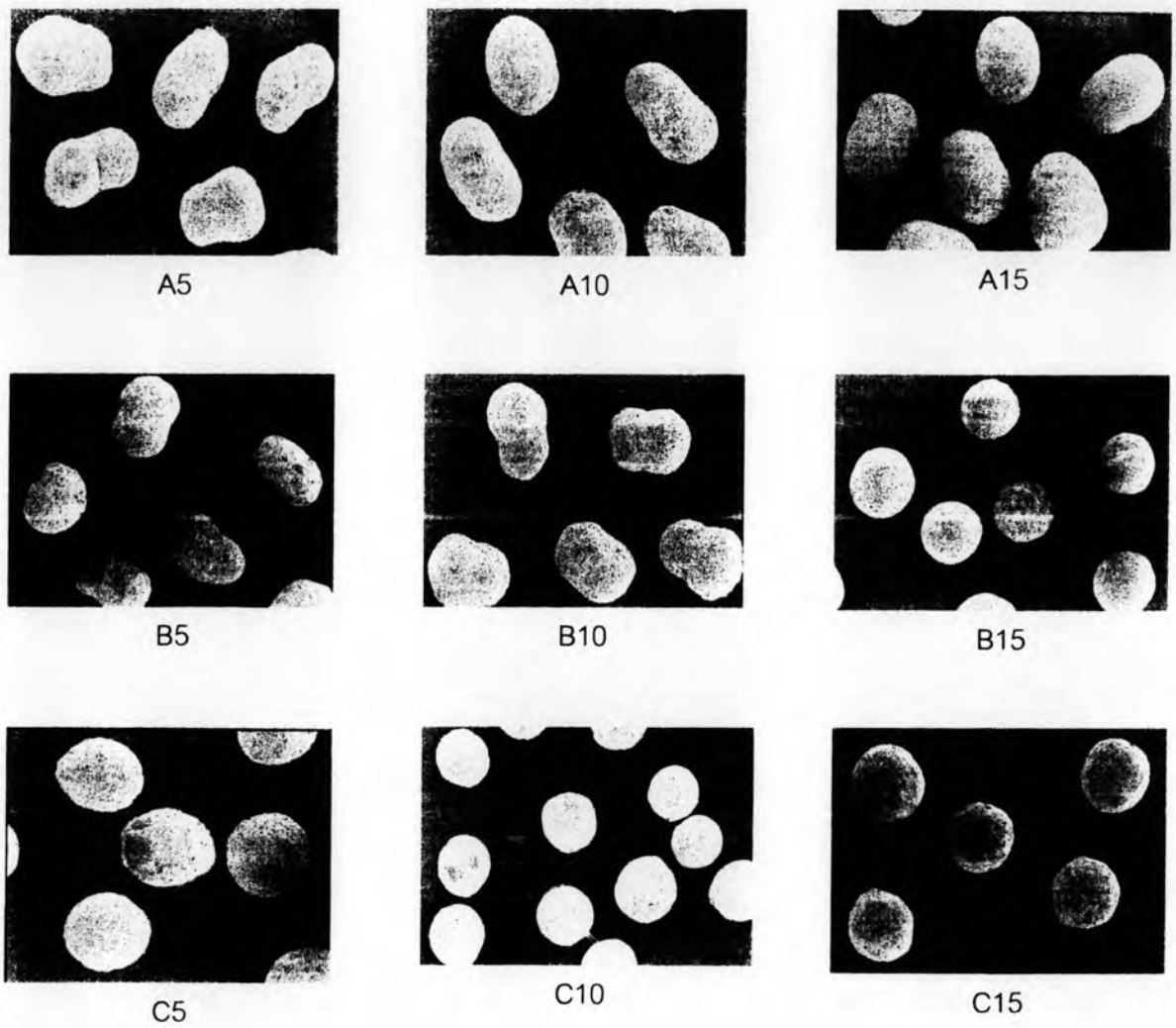


Figure 13 Photomicrographs of stavudine pellets (Formulation P5) prepared with various spheronization times and spheronizer speeds (×25)

(A5, A10, A15 are stavudine pellet used spheronizer speed of 400 rpm and spheronization times 5, 10, 15 mins ;

B5, B10, B15 are stavudine pellet used spheronizer speed of 600 rpm and spheronization times 5, 10, 15 mins ;

C5, C10, C15 are stavudine pellet used spheronizer speed of 860 rpm and spheronization times 5, 10, 15 mins)

Table 10 Aspect ratio of stavudine pellets prepared with various spheronization times and spheronization speeds

Formulations	Speronization Time (min)	Aspect ratio*		
		400 rpm	600 rpm	860 rpm
P1	5	1.42	1.30	1.28
	10	1.38	1.27	1.22
	15	1.25	1.23	1.17
P2	5	1.82	1.74	1.39
	10	1.71	1.66	1.34
	15	1.63	1.57	1.32
P3	5	1.87	1.79	1.45
	10	1.72	1.69	1.41
	15	1.66	1.61	1.38
P4	5	1.20	1.15	1.10
	10	1.15	1.11	1.08
	15	1.12	1.09	1.07
P5	5	1.25	1.23	1.21
	10	1.21	1.18	1.13
	15	1.16	1.12	1.09

*Results are averaged from two determinations (300 samples per one determination).

2. Particle Size Distribution

Particle size distribution of stavudine pellet are presented in Table 11 to 13 and Figure 14 to 18. It can be seen that most pellets in formulation P1, P2 and P3 were in the sieve size of 14-25 mesh range while the highest proportion of pellets in formulation P4 and P5 were in the sieve size of 20-25 mesh range. These results obviously indicated that stavudine pellet in formulation P4 and P5 which used Avicel® PH101 as a filler and no lactose in the formulation, had more narrow size distribution than formulation P1, P2 and P3 which used lactose as a filler. The effect of spheronization time and spheronizer speed on particle size distribution are described as following ;

2.1 Percent Sieve Fraction on 16/25 Mesh Cut Determination

The effect of spheronization time and spheronizer speed on percent sieve fraction on 16/25 mesh cut of stavudine pellet were studied and presented in Table 11 to 13.

At spheronizer speed of 400 rpm, The percent sieve fraction on 16/25 mesh cut of stavudine pellet was ranging from 38.13-92.61%. Percent sieve fraction on 16/25 mesh cut of Formulation P3 at spheronization time 15 minutes was lower than the others (38.13%) while percent sieve fraction on 16/25 mesh cut of Formulation P4 at spheronization time 10 minutes was higher than the others (92.61%). The order of percent sieve fraction on 16/25 mesh cut of each formulation was P4 > P5 > P1 > P2 > P3. It is noticeable that increasing spheronization time seemed not different in percent sieve fraction on 16/25 mesh cut of Formulation P1, P4 and P5. However, for Formulation P2 and P3, percent sieve fraction on 16/25 mesh cut was decreased when the spheronization time was increased as order; percent sieve fraction on 16/25 mesh cut of : 5 minutes > 10 minutes > 15 minutes.

At spheronizer speed of 600 rpm, The percent sieve fraction on 16/25 mesh cut of stavudine pellet was ranging from 39.89-93.07%. Percent sieve fraction on 16/25 mesh cut of Formulation P3 at spheronization time 15 minutes was lower than the others (39.89%) and percent sieve fraction on 16/25 mesh cut of Formulation P4 at spheronization time 10 minutes was higher than the others (93.07%). The order of percent sieve fraction on 16/25 mesh cut of each formulation was P4 $\geq$ P5 > P1 > P2 > P3. Increasing spheronization time seemed not different in percent sieve fraction on 16/25 mesh cut of Formulation P1, P4 and P5. However, for Formulation P2 and P3, percent sieve fraction on 16/25 mesh cut was decreased when the spheronization time was increased as order; percent sieve fraction on 16/25 mesh cut of : 5 minutes > 10 minutes > 15 minutes.

At spheronizer speed of 860 rpm, The percent sieve fraction on 16/25 mesh cut of stavudine pellet was ranging from 40.31-93.48%. Percent sieve fraction on 16/25 mesh cut of Formulation P3 at spheronization time 10 minutes was lower than the others (40.31%) and percent sieve fraction on 16/25 mesh cut of Formulation P4 at spheronization time 10 minutes was higher than the others (93.48%). The order of percent sieve fraction on 16/25 mesh cut of each formulation was P4 > P5 > P1 > P2 > P3. Increasing spheronization time seemed not different in percent sieve fraction on 16/25 mesh cut of Formulation P1, P4 and P5. However, for Formulation P2, percent sieve fraction on 16/25 mesh cut was decreased when the spheronization time was increased as order; percent sieve fraction on 16/25 mesh cut of : 5 minutes > 10 minutes > 15 minutes. For Formulation P3, percent sieve fraction on 16/25 mesh cut of ; 5 minutes > 15 minutes $\geq$ 10 minutes

All results above indicated that increasing spheronization time and spheronizer speed had effect on percent sieve fraction on 16/25 mesh cut especially in formulation 2 and 3.

Percent yield on 16/25 mesh cut of stavudine pellet using Avicel PH101 as a filler was very higher than stavudine pellet using lactose as a filler. It can be seen that approximately 75-95% of required pellets were obtained in formulation P4 and P5 whereas approximately 40-70% of required pellets were obtained in formulation P1, P2 and P3. At each spheronization time and spheronizer speed, percent yield on 16/25 mesh cut of Formulation P4 was higher than of the others.

These results corresponds with the former results which was explained that stavudine pellet formulation P1, P2 and P3 using stavudine combined with lactose have high binding property and caused lumps during the spheronization step. When spheronization time was increased, larger pellets were observed. This was because the extrudates were stick and aggregated to produce larger pellets. These results also can be explained by the fact that increasing particle size of pellets may decrease the desirable particle range and lead to low percent yield at 16/25 mesh cut.

Table 11 Sieve analysis of stavudine pellets prepared with various spheronization times and spheronizer speed of 400 rpm

Formulations	Speronization Time (min)	Percent weight retained on sieve sizes no.*						% sieve fraction on 16/25 mesh cut
		14	16	18	20	25	pan	
P1	5	5.48	20.21	13.35	20.97	28.19	11.80	62.51
	10	18.39	15.69	19.82	26.54	19.03	0.53	65.39
	15	20.41	18.16	17.48	25.25	18.41	0.29	61.14
P2	5	11.55	23.00	14.83	23.06	22.96	4.60	60.85
	10	20.63	28.17	20.26	17.32	13.24	0.38	50.82
	15	25.38	30.74	21.15	15.41	6.53	0.79	43.09
P3	5	20.32	24.17	15.26	20.47	18.39	1.72	54.12
	10	23.74	28.89	15.78	14.23	15.96	1.40	45.97
	15	29.91	31.53	14.37	15.59	8.17	0.43	38.13
P4	5	1.45	2.06	8.03	35.21	46.22	7.03	89.46
	10	2.67	1.02	5.99	48.78	37.84	3.70	92.61
	15	3.94	3.95	7.74	47.66	35.12	1.59	90.52
P5	5	4.23	4.68	9.02	36.75	40.88	4.44	86.65
	10	5.81	5.37	11.46	43.93	32.67	0.76	88.06
	15	6.12	7.29	16.54	41.11	28.09	0.85	85.74

* The results are averaged from three determinations.

Table 12 Sieve analysis of stavudine pellets prepared with various spheronization times and spheronizer speed of 600 rpm

Formulations	Speronization Time (min)	Percent weight retained on sieve sizes no.*						% sieve fraction on 16/25 mesh cut
		14	16	18	20	25	pan	
P1	5	4.07	19.13	12.88	20.25	31.41	12.26	64.54
	10	16.89	13.26	19.05	28.93	20.75	1.12	68.73
	15	19.69	17.48	16.31	26.22	19.93	0.37	62.46
P2	5	11.32	22.57	13.84	22.96	23.38	5.93	60.18
	10	20.15	25.94	20.08	17.07	14.49	2.27	51.64
	15	23.78	29.56	19.29	18.85	7.43	1.09	45.57
P3	5	18.11	23.08	14.79	20.96	19.62	3.44	55.37
	10	22.56	27.49	14.80	16.74	16.23	2.18	47.77
	15	28.45	30.72	15.04	18.27	6.58	0.94	39.89
P4	5	0.97	1.75	7.66	33.18	45.35	11.09	86.19
	10	1.21	0.89	5.38	48.45	39.24	4.83	93.07
	15	2.54	3.26	7.32	46.09	37.16	3.63	90.57
P5	5	3.09	3.95	9.04	34.63	39.20	10.09	82.87
	10	4.67	5.40	11.19	41.29	33.99	3.46	86.47
	15	5.28	7.07	15.78	39.74	31.05	1.08	86.57

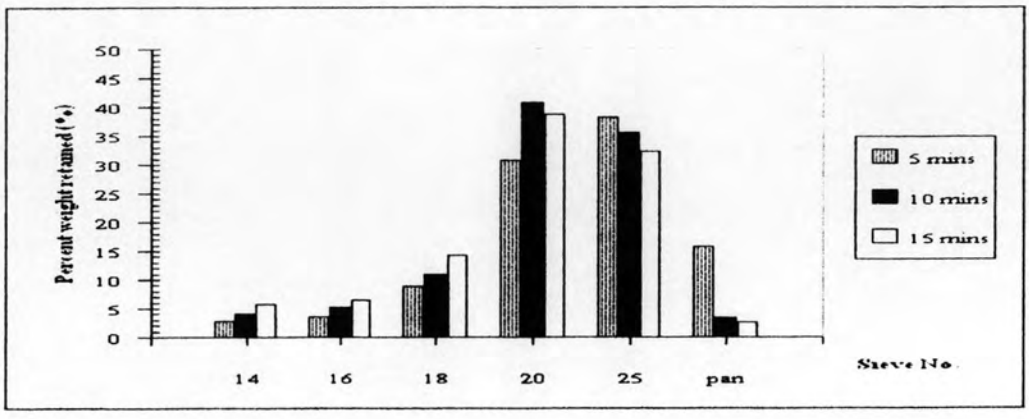
* The results are averaged from three determinations.

Table 13 Sieve analysis of stavudine pellets prepared with various spheronization times and spheronizer speed of 860 rpm

Formulations	Speronization Time (min)	Percent weight retained on sieve sizes no.*						% sieve fraction on 16/25 mesh cut
		14	16	18	20	25	pan	
P1	5	3.69	18.81	12.34	20.98	32.23	11.96	65.55
	10	15.12	12.58	18.72	29.31	22.15	2.12	70.18
	15	18.55	15.03	15.28	27.17	21.69	2.28	64.14
P2	5	10.87	21.48	13.39	20.23	23.62	4.41	57.24
	10	20.75	24.62	18.47	16.89	15.54	3.73	50.9
	15	22.19	28.12	18.34	20.15	10.04	1.16	48.53
P3	5	17.54	22.9	13.33	21.81	20.44	3.98	55.58
	10	20.35	26.43	10.29	17.84	12.18	2.91	40.31
	15	25.56	30.72	15.47	19.68	7.53	1.04	42.68
P4	5	0.23	1.58	7.19	30.06	44.72	16.22	81.97
	10	0.42	0.86	4.68	47.93	40.87	5.24	93.48
	15	2.76	3.09	7.15	44.36	38.92	3.72	90.43
P5	5	2.87	3.49	8.96	30.72	38.15	15.81	77.83
	10	4.06	5.25	10.97	40.75	35.63	3.34	87.35
	15	5.78	6.43	14.32	38.69	32.14	2.64	85.15

* The results are averaged from three determinations.

A.



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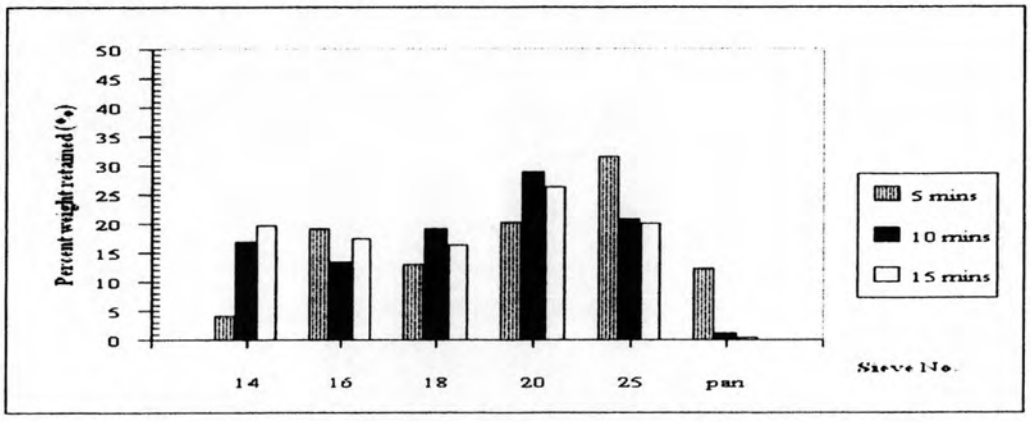

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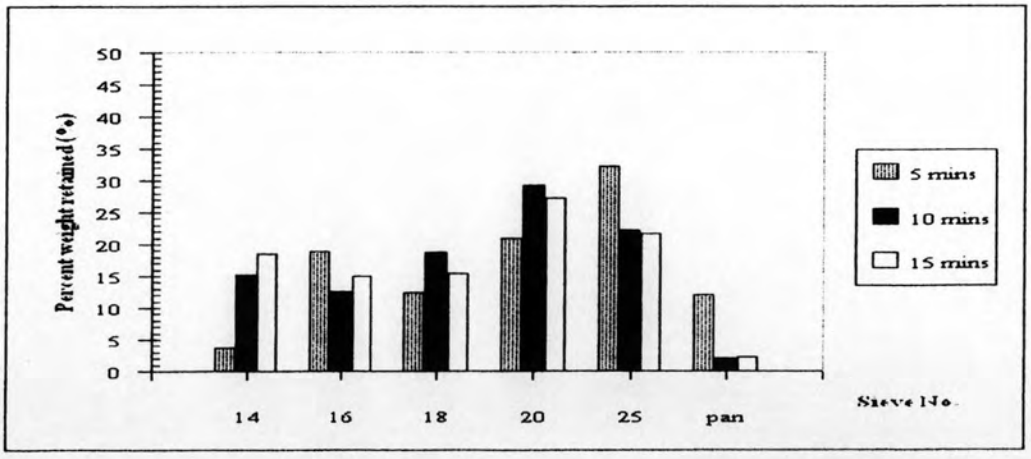


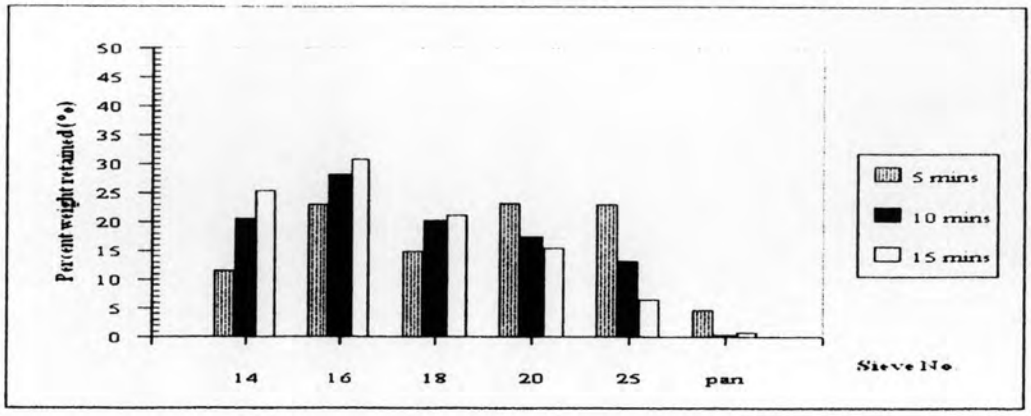
Figure 14 Percentage amount of stavudine pellets (Formulation P1) prepared with various spheronization times, retained on sieves

A : Spheronizer speed of 400 rpm

B : Spheronizer speed of 600 rpm

C : Spheronizer speed of 860 rpm

A.



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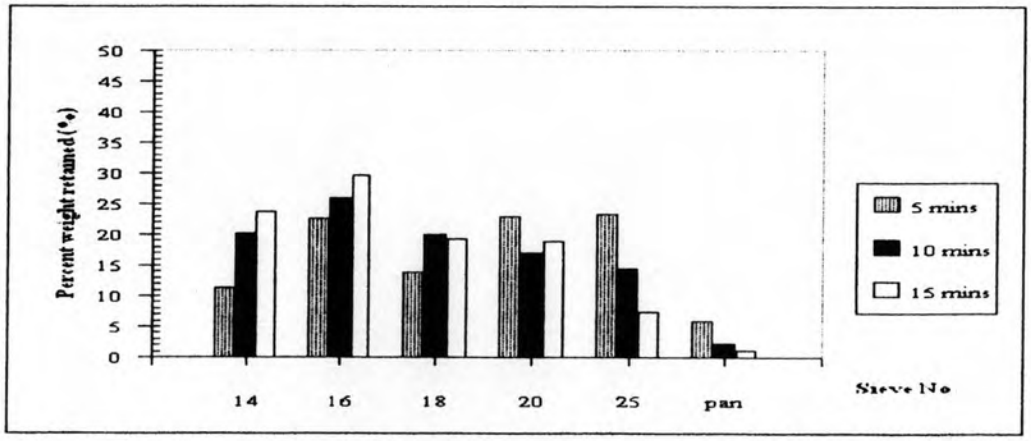

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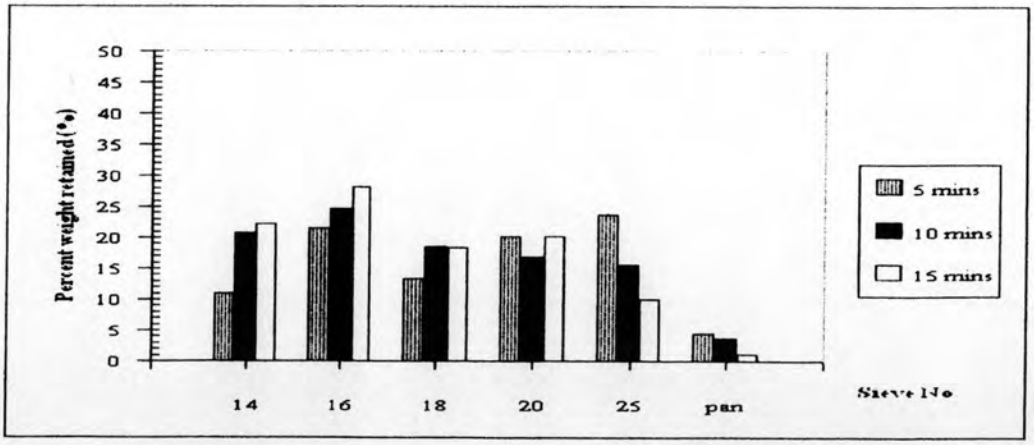


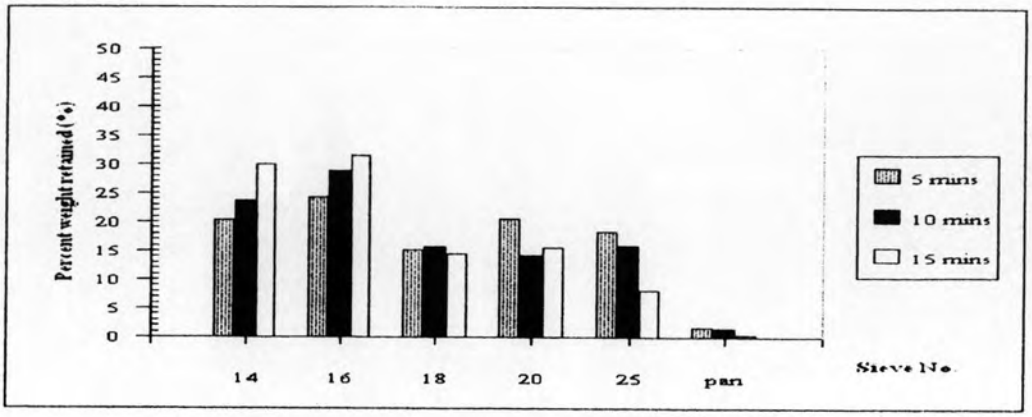
Figure 15 Percentage amount of stavudine pellets (Formulation P2) prepared with various spheronization times, retained on sieves

A : Spheronizer speed of 400 rpm

B : Spheronizer speed of 600 rpm

C : Spheronizer speed of 860 rpm

A.



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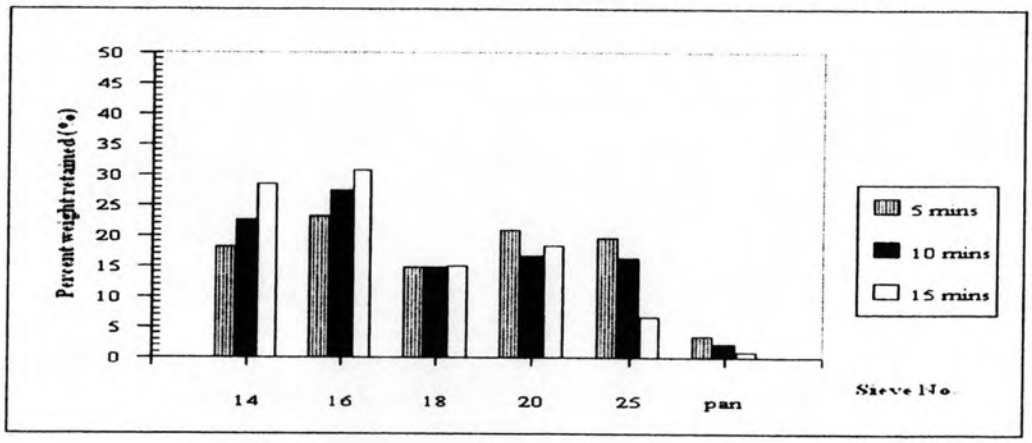

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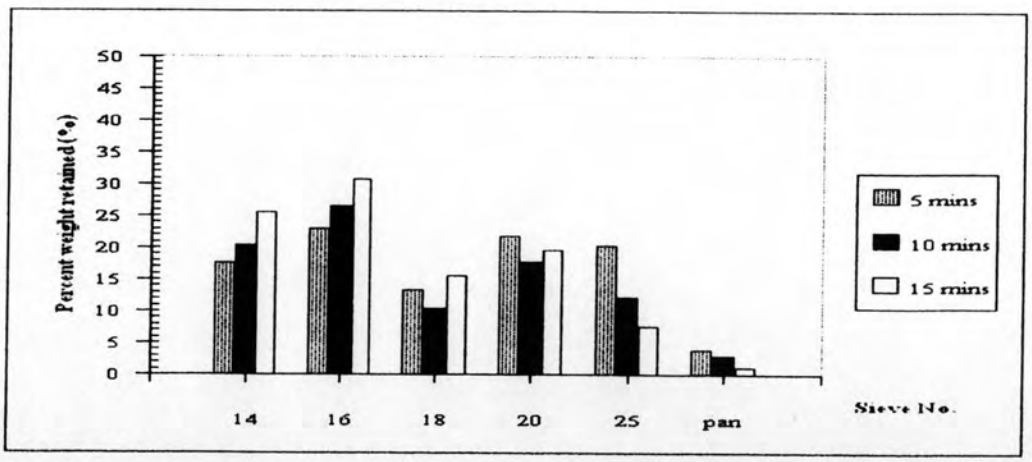


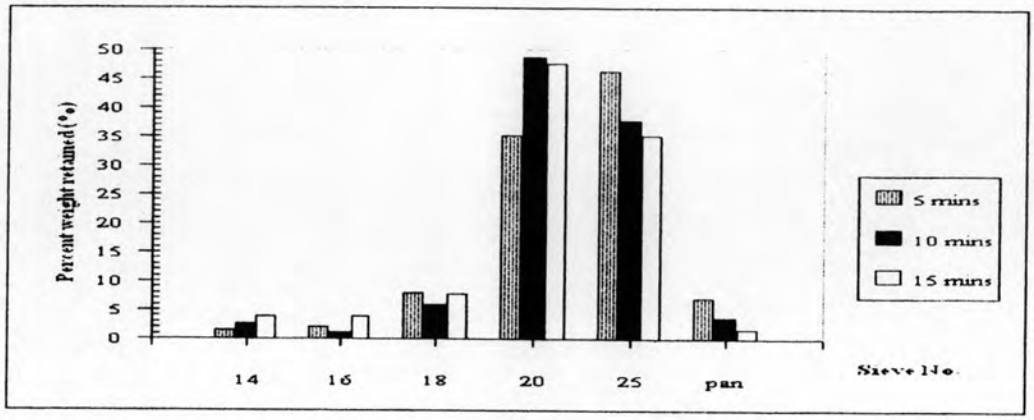
Figure 16 Percentage amount of stavudine pellets (Formulation P3) prepared with various spheronization times, retained on sieves

A : Spheronizer speed of 400 rpm

B : Spheronizer speed of 600 rpm

C : Spheronizer speed of 860 rpm

A.



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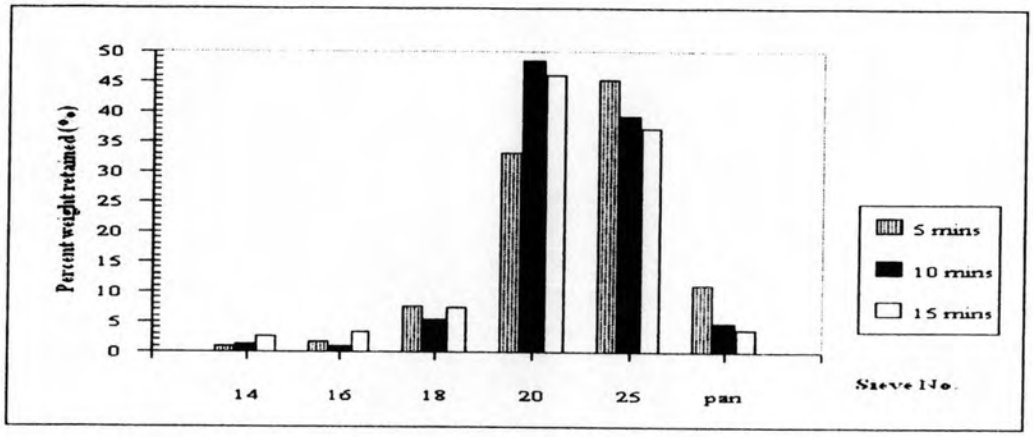


C.

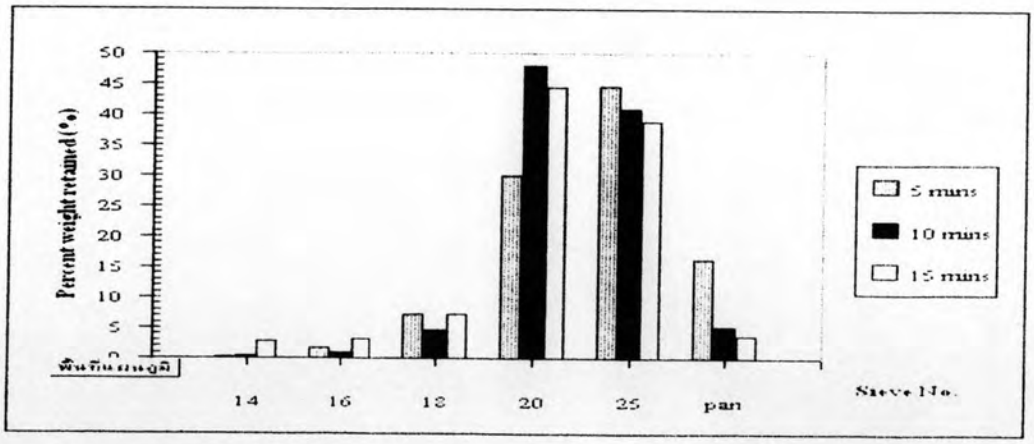


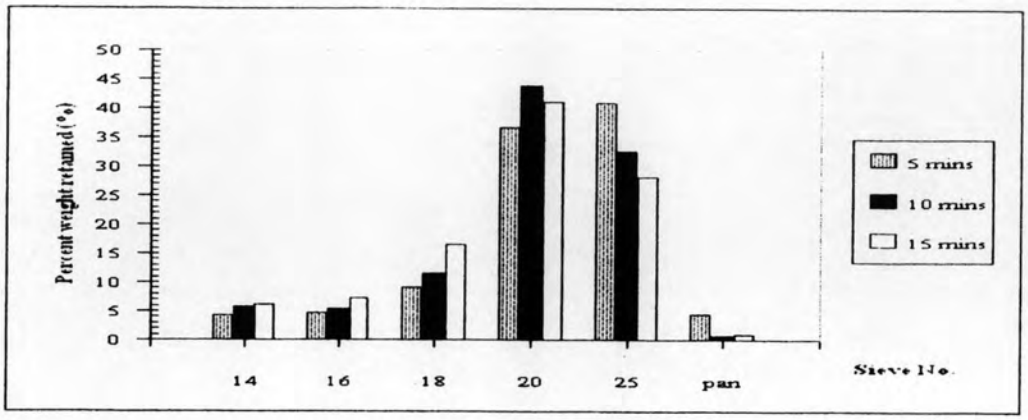
Figure 17 Percentage amount of stavudine pellets (Formulation P4) prepared with various spheronization times, retained on sieves

A : Spheronizer speed of 400 rpm

B : Spheronizer speed of 600 rpm

C : Spheronizer speed of 860 rpm

A.



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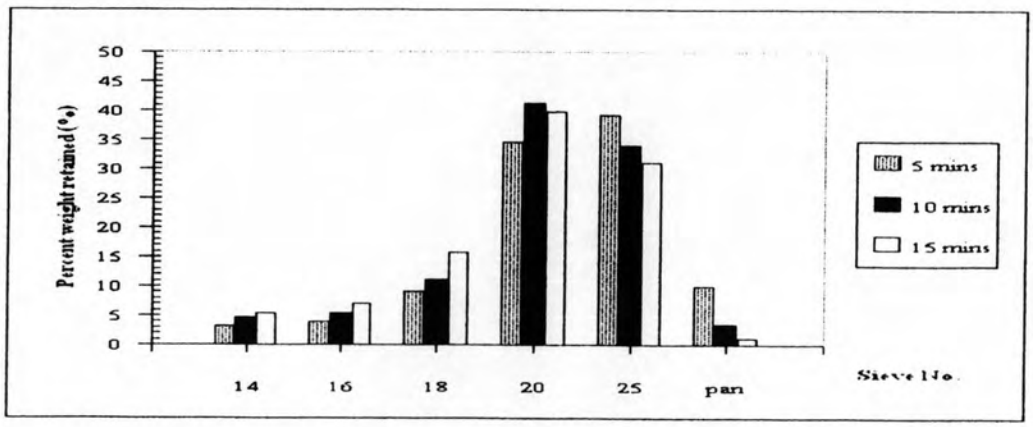

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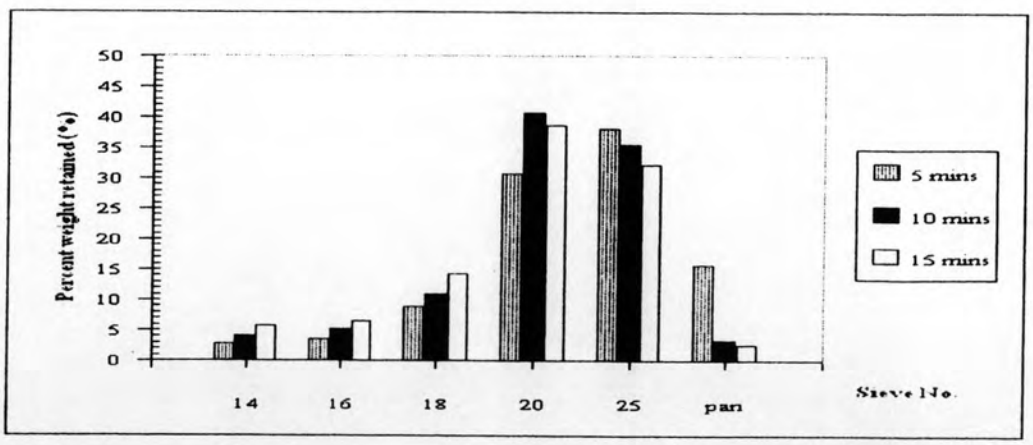


Figure 18 Percentage amount of stavudine pellets (Formulation P5) prepared with various spheronization times, retained on sieves

A : Spheronizer speed of 400 rpm

B : Spheronizer speed of 600 rpm

C : Spheronizer speed of 860 rpm

2.2 Median Particle Size

The effect of spheronization time and spheronizer speed on the median particle size of stavudine pellet are shown in Table 14. The median particle size of stavudine pellet were ranging from 0.81-1.26 mm. At the same spheronizer speed and spheronization time, the order of median particle size of pellets in each formulation were as follow : formulation P3 > formulation P2 > formulation P1 > formulation P5 > formulation P4.

The median particle size of pellets were varied in different spheronization time and spheronization speed. Mean particle size of pellets was increased with increasing spheronization time. At each spheronizer speed, the order of median particle size of pellets were as follow : 15 minutes > 10 minutes > 5 minutes. When spheronization time was increased, pellets combined with the fine particles that occurred in the process. These results may be explained by the fact that changing of median particle size occured with increasing spheronization time. Therefore, %yield of pellets on sieve fraction 16/25 mesh cut was changed by various spheronization times.

Increasing spheronization speed had little effect on the median particle size. At each spheronization time, the order of median particle size of pellets were as follow : 400 rpm .> 600 rpm > 860 rpm.

Table 14 Median particle size of stavudine pellet prepared with various spheronization times and spheronization speeds

Formulations	Speronization Time (min)	Median particle size (mm)*		
		400 rpm	600 rpm	860 rpm
P1	5	0.92	0.89	0.88
	10	1.04	1.00	0.98
	15	1.06	1.04	0.99
P2	5	1.00	0.98	0.97
	10	1.17	1.14	1.13
	15	1.22	1.20	1.18
P3	5	1.11	1.07	1.05
	10	1.20	1.18	1.12
	15	1.26	1 25	1.22
P4	5	0.83	0.82	0.81
	10	0.87	0.86	0.85
	15	0.88	0.87	0.87
P5	5	0.86	0.84	0.83
	10	0.90	0.89	0.88
	15	0.92	0.91	0.90

*The results are averaged from two determinations

3. **Bulk Density, Tapped Density and %Carr's compressibility Determination**

The effect of spheronization time and spheronization speed on bulk density and tapped density of stavudine pellet are presented in Table 15.

Bulk density and tapped density of stavudine pellet were varied in different spheronization speed and spheronization time. However, increasing spheronization speed and spheronization time had not effect on bulk density and tapped density of stavudine pellet.

Bulk density and tapped density of stavudine pellet obtained from formulation P4 and P5 were higher than bulk density and tapped density of stavudine pellet obtained from Formulation P1, P2 and P3. However, no difference between bulk density and tapped density for formulation P4 and P5. The reason may be due to pellets in formulation P4 and P5 had more narrow size distribution and the mean particle size was smaller than pellets in formulation P1, P2 and P3. Therefore, smaller pellets obtained from formulation P4 and P5 can probably arrange in closest packing. This result also corresponsed with Funk, et al. (1991)

By calculation, %Carr's compressibility of stavudine pellet obtained from formulation P4 and P5 were lower than %Carr's compressibility of stavudine pellet in Formulation P1, P2 and P3. These results reflected a good compressibility of stavudine pellet using Avicel PH101® in the formulation.

Table 15 Bulk density, Tapped density and % Carr's compressibility of stavudine pellets prepared with various spheronization times and spheronization speeds*

Speed (rpm)	Speronization Time (min)	Bulk density (g/ml ± SD)					Tapped density (g/ml ±SD)					%Carr's compressibility				
		P1	P2	P3	P4	P5	P1	P2	P3	P4	P5	P1	P2	P3	P4	P5
400	5	0.73 (0.02)	0.73 (0.01)	0.72 (0.01)	0.86 (0.01)	0.86 (0.01)	0.76 (0.03)	0.76 (0.02)	0.75 (0.01)	0.87 (0.01)	0.87 (0.00)	3.95	3.95	4.00	1.15	1.15
	10	0.73 (0.01)	0.73 (0.01)	0.71 (0.01)	0.86 (0.00)	0.85 (0.01)	0.77 (0.02)	0.76 (0.01)	0.74 (0.01)	0.87 (0.01)	0.86 (0.01)	5.19	3.95	4.05	1.15	1.16
	15	0.72 (0.01)	0.71 (0.01)	0.71 (0.02)	0.85 (0.01)	0.85 (0.01)	0.76 (0.02)	0.75 (0.01)	0.75 (0.01)	0.86 (0.01)	0.86 (0.01)	5.26	5.33	5.33	1.16	1.16
600	5	0.73 (0.02)	0.73 (0.01)	0.72 (0.01)	0.85 (0.00)	0.85 (0.01)	0.75 (0.02)	0.75 (0.01)	0.74 (0.01)	0.86 (0.01)	0.87 (0.01)	2.67	2.67	2.70	1.16	2.30
	10	0.74 (0.01)	0.71 (0.01)	0.71 (0.02)	0.84 (0.01)	0.84 (0.01)	0.76 (0.01)	0.73 (0.01)	0.73 (0.01)	0.85 (0.01)	0.85 (0.01)	2.63	2.74	2.74	1.18	1.18
	15	0.73 (0.01)	0.71 (0.02)	0.71 (0.02)	0.84 (0.01)	0.83 (0.00)	0.75 (0.02)	0.73 (0.02)	0.73 (0.01)	0.84 (0.01)	0.84 (0.01)	2.67	2.74	2.74	0.00	1.19
860	5	0.75 (0.01)	0.74 (0.00)	0.83 (0.02)	0.85 (0.00)	0.84 (0.00)	0.76 (0.01)	0.76 (0.01)	0.75 (0.01)	0.85 (0.01)	0.85 (0.01)	1.32	2.63	2.67	0.00	1.18
	10	0.74 (0.01)	0.73 (0.01)	0.83 (0.01)	0.84 (0.01)	0.84 (0.01)	0.76 (0.01)	0.75 (0.01)	0.75 (0.01)	0.85 (0.00)	0.85 (0.01)	2.63	2.67	2.67	1.18	1.18
	15	0.74 (0.01)	0.72 (0.01)	0.82 (0.01)	0.83 (0.01)	0.84 (0.01)	0.76 (0.02)	0.74 (0.01)	0.74 (0.01)	0.84 (0.02)	0.85 (0.01)	2.63	2.70	2.70	1.19	1.18

*Average from three determinations.

4. Flow rate Determination

Flow rate of stavudine pellet prepared with various spheronization times and spheronization speeds are presented in Table 16. The range of flow rate was between 240.27-294.46 g/min.

Flow rate was increased with increasing spheronizer speed. Ranging of flow rate as a function of spheronization speed were following : 860 rpm > 600 rpm > 400 rpm.

At the same spheronization speed, flow rate was increased with increasing spheronization time from 5 to 10 minutes (at 10 minutes the flow rate was higher than that of the others), after that flow rate would be decreased at spheronization time 15 minutes.

Flow rate of stavudine pellet obtained from formulation P4 and P5 were higher than flow rate of stavudine pellet obtained from Formulation P1, P2 and P3. This can be explained that pellets in formulation P4 and P5 had more narrow size distribution and shape of pellets was more spherical than pellets in formulation P1, P2 and P3. Therefore, pellets obtained from formulation P4 and P5 have better flowability than that of formulation P1, P2 and P3.

In this study, pellets obtained from formulation P4 at spheronizer speed 860 rpm and spheronization time 10 minutes gave the maximum flow rate (294.46 g/min).

These results indicated that flow rate of pellets varied by spheronizer speed and spheronization time. However, the range of flow rate for all pellets indicated good flowability.

Table 16 Flow rate of stavudine pellets prepared with various spheronization times and spheronization speeds

Speed (rpm)	Spheronization Time (min)	Flow Rate (g/min)*				
		P1	P2	P3	P4	P5
400	5	250.49	243.78	241.24	273.2	272.87
	10	257.82	252.65	248.76	285.61	288.84
	15	246.64	246.44	236.81	275.36	275.44
600	5	253.65	244.82	248.36	276.34	274.68
	10	264.37	253.05	251.03	281.26	283.53
	15	256.48	249.56	240.27	275.61	270.13
860	5	261.33	251.37	246.89	282.78	279.94
	10	267.98	256.76	251.02	294.46	283.15
	15	258.42	248.18	243.47	285.62	271.41

*The results are averaged from three determinations

5. Angle of repose Determination

Angle of repose of stavudine pellet prepared with various spheronization times and spheronization speeds are presented in Table 17. The range of angle of repose was between 23.10-29.92^0.

All spheronization times and spheronization speeds were studied. Angle of repose of stavudine pellet were varied in different spheronization speed and spheronization time. However, increasing spheronization speed and spheronization time had not effect on angle of repose of stavudine pellet.

It was observed that angle of repose of stavudine pellet obtained from formulation P4 and P5 were lower than angle of repose of stavudine pellet obtained from Formulation P1, P2 and P3. This result complied with the result of flow rate which indicated that stavudine pellet obtained from formulation P4 and P5 have better flowability than that of formulation P1, P2 and P3. However, because the range of angle of repose for all formulations was low, thus it indicated that all formulations have good flowability.

In this study, pellets obtained from formulation P4 at spheronizer speed 860 rpm and spheronization time 10 minutes gave the minimum angle of repose (23.10^0).

6. Percentage Friability determination

The effect of spheronization time and spheronization speed on percentage friability of stavudine pellets are presented in Table 18.

For all case, the percentage friability was ranging from 0.1016 to 0.1774. This result indicated that all formulations had very low friability.

Percentage friability of stavudine pellets were varied in different spheronization speeds and spheronization times. However, spheronization speed and spheronization time were not significantly affected on the percentage friability of stavudine pellets.

Table 17 Angle of repose of stavudine pellets prepared with various spheronization times and spheronization speeds

Speed (rpm)	Spheronization Time (min)	Angle of repose (degree)*				
		P1	P2	P3	P4	P5
400	5	28.32	29.36	29.45	24.68	24.88
	10	27.67	28.54	29.01	23.45	23.69
	15	28.12	28.97	29.76	24.31	24.05
600	5	28.45	29.43	29.92	24.52	24.74
	10	27.98	28.38	28.23	23.40	24.02
	15	27.30	28.61	28.82	23.96	24.27
860	5	27.53	29.25	29.57	23.65	24.13
	10	26.74	27.46	28.16	23.10	23.28
	15	27.14	28.72	28.85	23.26	23.63

*Averaged from three determinations.

Table 18 Percentage friability of stavudine pellets prepared with various spheronization times and spheronization speeds

Speed (rpm)	Spheronization Time (min)	%Friability*				
		P1	P2	P3	P4	P5
400	5	0.1892	0.1457	0.1228	0.1737	0.1676
	10	0.1774	0.1339	0.1243	0.1652	0.1564
	15	0.1736	0.1342	0.1052	0.1608	0.1539
600	5	0.1861	0.1317	0.1269	0.1797	0.1238
	10	0.1769	0.1161	0.1203	0.1552	0.1105
	15	0.1743	0.1175	0.1041	0.1648	0.1067
860	5	0.1755	0.1339	0.1198	0.1694	0.1336
	10	0.1692	0.1248	0.1072	0.1588	0.1241
	15	0.1533	0.1387	0.1016	0.1563	0.1683

*The results are averaged from three determinations

7. Evaluation physical properties of uncoated & coated stavudine pellet

The physical properties of uncoated stavudine pellets obtained from formulation P4 at spheronizer speed 860 rpm and spheronization time 10 minute are summarized in Table 19.

The results indicated that pellets obtained from formulation P4 at spheronizer speed of 860 rpm and spheronization time 10 minute were relatively sphere with aspect ratio close to 1. Pellets had narrow size distribution, high percentage sieve fraction on 16/25 mesh cut, good flowability with high flow rate and low angle of repose and low percentage friability. It was also found that no difference between bulk density and tapped density were detected.

From all results, uncoated stavudine pellet obtained from formulation P4 consisted of stavudine 40% and Avicel® PH101 60% with spheronizer speed at 860 rpm and spheronization time 10 minute found to have good physical properties for coating process. Therefore, this formulation were chosen for preparing coated pellets in the next step.

The microscopic appearance of uncoated and coated stavudine pellet are present in Figure 19. Uncoated and coated stavudine pellet appeared to be round shape with fairly smooth surface. Thickness of coating layer on the pellets was smooth and consistent.

Table 19 Summary of physical properties of uncoated stavudine pellets obtained from formulation P4 at spheronizer speed 860 rpm and spheronization time 10 minutes.

Physical Properties	
Aspect ratio	1.08
% Sieve fraction on 16/25 mesh cut	93.48
Mean particle size (mm)	0.85
Bulk density (g/ml ± SD)	0.84 ± 0.01
Tapped density (g/ml ±SD)	0.85 ± 0.00
%Carr's compressibility	1.18
Flow Rate (g/min)	294.46
Angle of repose (degree)	23.10
%Friability	0.1588

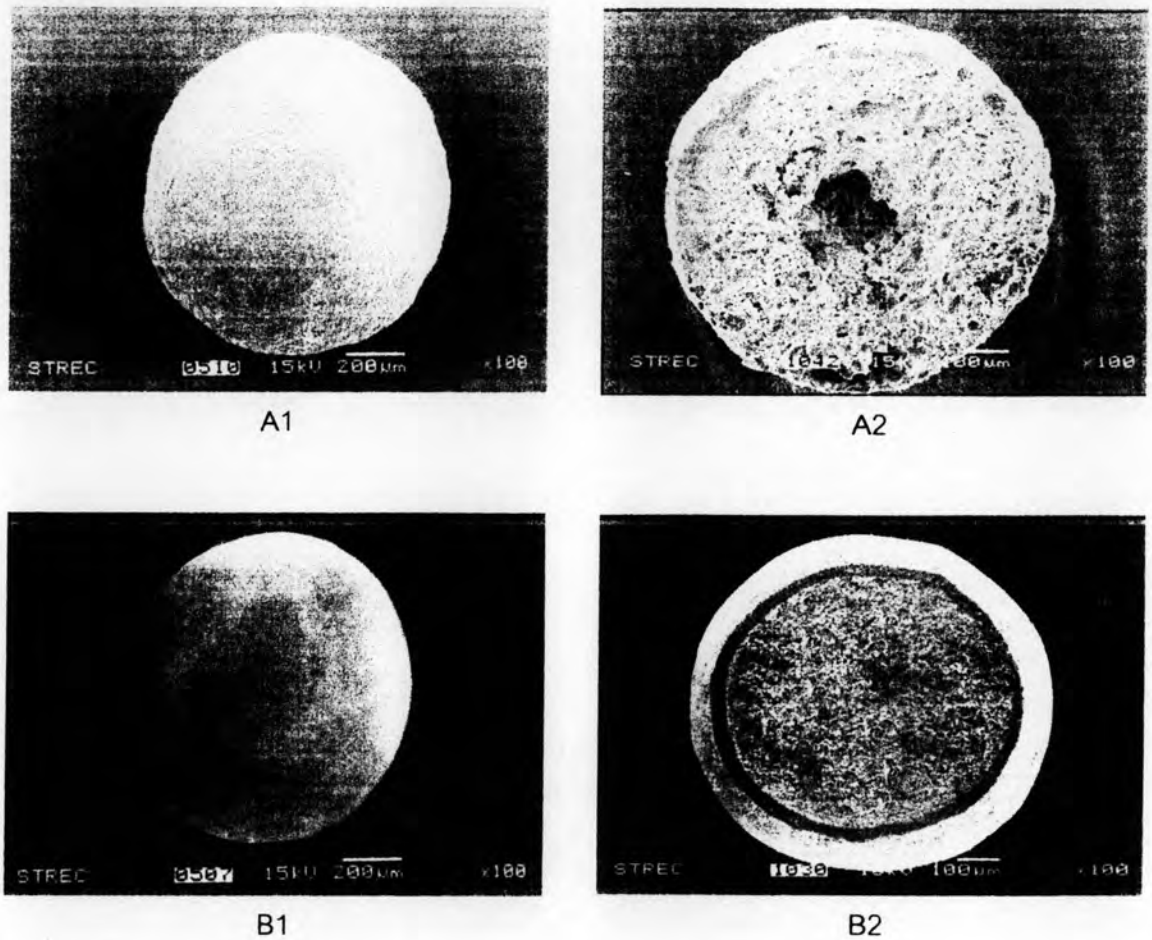


Figure 19 Photomicrographs of stavudine pellets (Formulation P4) (×100)
(A1, A2 are uncoated stavudine pellets prepared at spheronizer speed 860 rpm and spheronization time 10 min with over view and cross section ; B1, B2 are coated stavudine pellets with over view and cross section)

8. **Content uniformity of uncoated stavudine pellet**

Uncoated stavudine pellet (Formulation P4) were tested for uniformity of dosage unit. The results are presented in Table 20. All of them had stavudine content within the range of 85.0-115.0% label amount and percentage relative standard deviation (R.S.D.) was less than 6.0%. This result indicated that the manufacturing process by extrusion-spheronization could provide content uniformity of dosage form.

Table 20 Content uniformity of Dosage Units of uncoated stavudine pellets

Sample no.	%Labeled Amount
1	99.75
2	103.42
3	100.27
4	99.48
5	101.53
6	102.76
7	102.91
8	101.84
9	99.87
10	103.52
Mean	101.54
S.D.	1.59
% R.S.D	1.57

9. Assay content for active ingredient of pellets

Both uncoated and coated stavudine pellet were assayed for content of active ingredient. The results are shown in Table 21. All of them had stavudine content within the range of 85.0-115.0% label amount and relative standard deviation (R.S.D.) was less than 6.0%. This result indicated that no indications of a reduction in active ingredient during extrusion-spheronization and coating process.

Table 21 Assay for content of active Ingredient in core pellets and coated pellets

Sample no.	% Labeled Amount	
	Core pellet	Coated pellet
1	102.32	101.64
2	101.45	100.96
3	100.92	99.63
Mean	101.56	100.74
S.D.	0.71	1.02
% R.S.D	0.70	1.01

10. In-vitro Analytical Method Validation

10.1 Linearity and Range

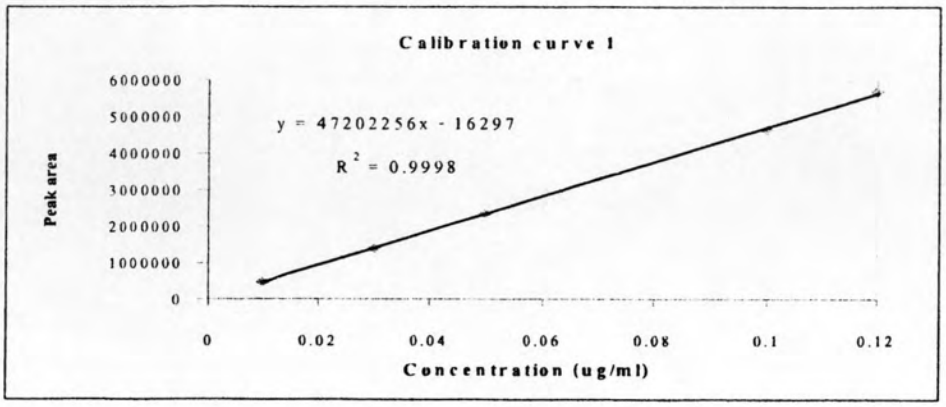
The linearity was determined from triplicate of five levels of calibration curves which plotted between peak area and stavudine concentrations. Linearity was observed within the concentration ranges of 0.01-0.12 mg/ml as shown in Table 22 and Figure 20.

Three standard calibration curves showed linear response over the range of concentrations used in the assay procedure. Linear regressions of peak area versus concentration gave a typical coefficient of determination (R^2) of 0.9998, 0.9999, 1.0000. These results were within the acceptance criteria.

Table 22 Linearity and Range of HPLC Analytical Method for Determination of Stavudine

Conc. of stavudine (mg/ml)	Peak area		
	1	2	3
0.01	465065	449110	444525
0.03	1394392	1395802	1383016
0.05	2350536	2291215	2314288
0.10	4653241	4543808	4604188
0.12	5687979	5514520	5499715
R^2	0.9998	0.9999	1.0000

A.



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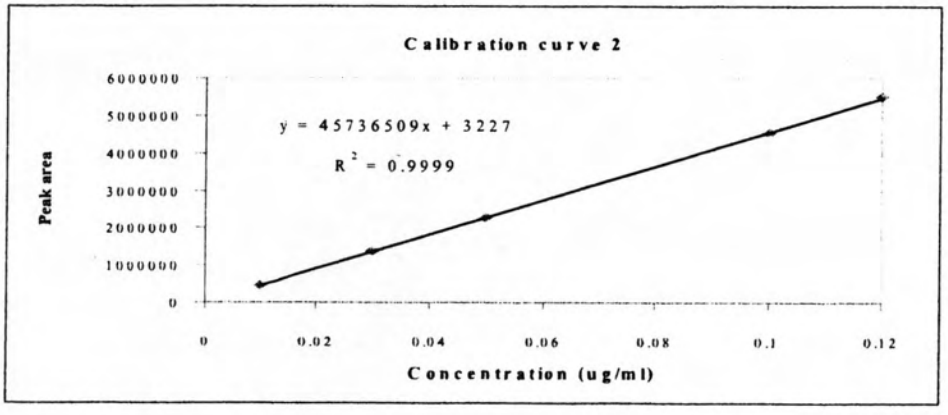

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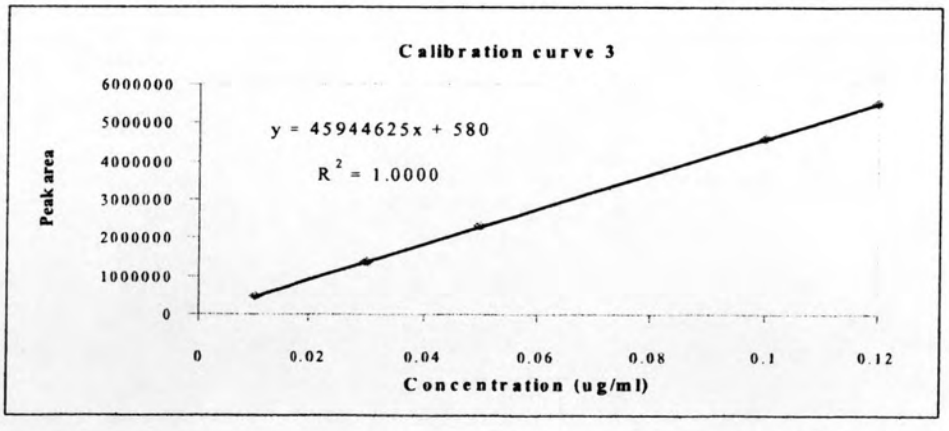


Figure 20 Calibration curves of Stavudine

(A = Calibration curve 1,

B = Calibration curve 2,

C = Calibration curve 3)

10.2 Limit of detection (LOD) & Limit of quantification (LOQ)

Limit of detection (LOD) and limit of quantification (LOQ) were determined by HPLC from stavudine concentration ranges of 0.001-0.030 μg/ml.

The signal to noise ratio of each concentration are shown in Table 23. The signal to noise ratio of stavudine concentration 0.002 μg/ml and 0.025 μg/ml were 2.8 and 10.18, respectively. Therefore, the limit of detection (LOD) and limit of quantification (LOQ) of the analytical method for determination of Stavudine were 0.002 μg/ml and 0.025 μg/ml, respectively.

Table 23 Limit of detection (LOD) and Limit of quantification (LOQ) of HPLC Analytical Method for Determination of Stavudine

Conc. of stavudine (μg/ml)	Signal-to-noise ratio
0.001	1.20
0.002	2.80
0.003	3.82
0.020	7.77
0.025	10.18
0.030	13.74

10.3 Accuracy and Precision

The accuracy, within- and between-run precision of the analytical method for determination of stavudine were assesses by analyzing quality control samples spiked with known amount of stavudine. The results are shown in Table 24 - 26, respectively.

It was observed that percent recovery of stavudine were between 98.78 to 101.20%. Standard deviation (S.D.) for within-run precision of stavudine was 0.0006 and they were between 0.0006-0.0007 for between-run precision. The percent coefficient of variation (%C.V.) for within-run precision of stavudine was 1.19 % and they were between 1.18-1.38 % for between-run precision.

These results were within acceptance criteria for accuracy (%recovery was between $\pm$ 15%) and precision (S.D. and % C.V. < 2%). The method showed to be precise and accurate.

Table 24 Accuracy of HPLC Analytical Method for Determination of Stavudine

Sample No.	Known conc. (mg/ml)	Estimated conc. (mg/ml)	%Recovery
1	0.01	0.0099	99.33
2	0.03	0.0296	98.78
3	0.05	0.0506	101.20
4	0.10	0.1011	101.10
5	0.12	0.1197	99.78

Table 25 Within-run Precision of HPLC Analytical Method for Determination of Stavudine

Sample No.	Known conc. (mg/ml)	Estimated conc. (mg/ml)
1	0.05	0.0508
2	0.05	0.0512
3	0.05	0.0514
4	0.05	0.0498
5	0.05	0.0505
6	0.05	0.0507
Average		0.0506
S.D.		0.0006
% C.V.		1.19

Table 26 Between-run Precision of HPLC Analytical Method for Determination of Stavudine

Sample	Day 1		Day 2		Day 3	
	Known Conc. (mg/ml)	Estimated Conc. (mg/ml)	Known Conc. (mg/ml)	Estimated Conc. (mg/ml)	Known Conc. (mg/ml)	Estimated Conc. (mg/ml)
1	0.05	0.0508	0.05	0.0511	0.05	0.0503
2	0.05	0.0512	0.05	0.0506	0.05	0.0511
3	0.05	0.0514	0.05	0.0509	0.05	0.0514
4	0.05	0.0498	0.05	0.0498	0.05	0.0515
5	0.05	0.0505	0.05	0.0510	0.05	0.0498
6	0.05	0.0507	0.05	0.0522	0.05	0.0504
Mean	0.0506		0.0509		0.0508	
S.D.	0.0006		0.0007		0.0006	
%C.V.	1.19		1.38		1.18	

11. Dissolution result

11.1 Dissolution Profiles of Uncoated stavudine pellets and Zerit® IR

The dissolution profiles of uncoated stavudine pellets and Zerit® IR which were plotted between the percentage amount of drug released as a function of time are presented in Figure 21 to 22 and dissolution data are present in Appendix C.

The results indicated that the release of stavudine from uncoated stavudine pellets and Zerit® IR in water, 0.1 N HCl, phosphate buffer pH 6.8 were very fast. In all dissolution mediums, at least 90% of stavudine were released within 15 minutes. The reason is the fact that, stavudine is a highly soluble drug even in water, acid or basic medium. Uncoated stavudine pellets without coating barrier, a very fast influx of medium occurred then the dissolution drug outflowed into the medium through the concentration gradient.

It was observed that after stavudine was completely released in the first hour, stavudine content in dissolution medium was continually decreased as shown that at 24 hours content of stavudine in 0.1 N HCl was less than those in phosphate buffer pH 6.8 and in water, respectively. In this study it was determined by HPLC that when the time which product was dissolved in dissolution medium increased, peak response of the degradation compound was increased. The degradation compound of stavudine detected was thymine. These results can be explained by Ashenafi Dunge et al (2005) who studied about stability of stavudine and found that stavudine can be hydrolysed under acidic, neutral and alkaline conditions and the major degradation product was thymine.

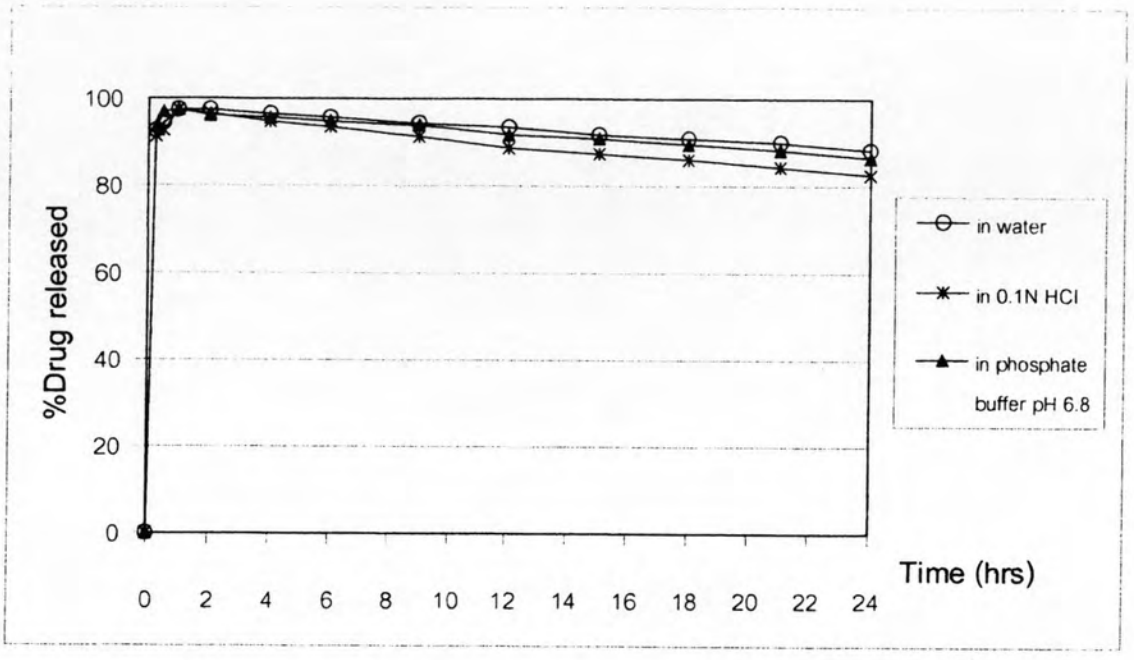


Figure 21 Dissolution profiles of uncoated stavudine pellets in different dissolution mediums

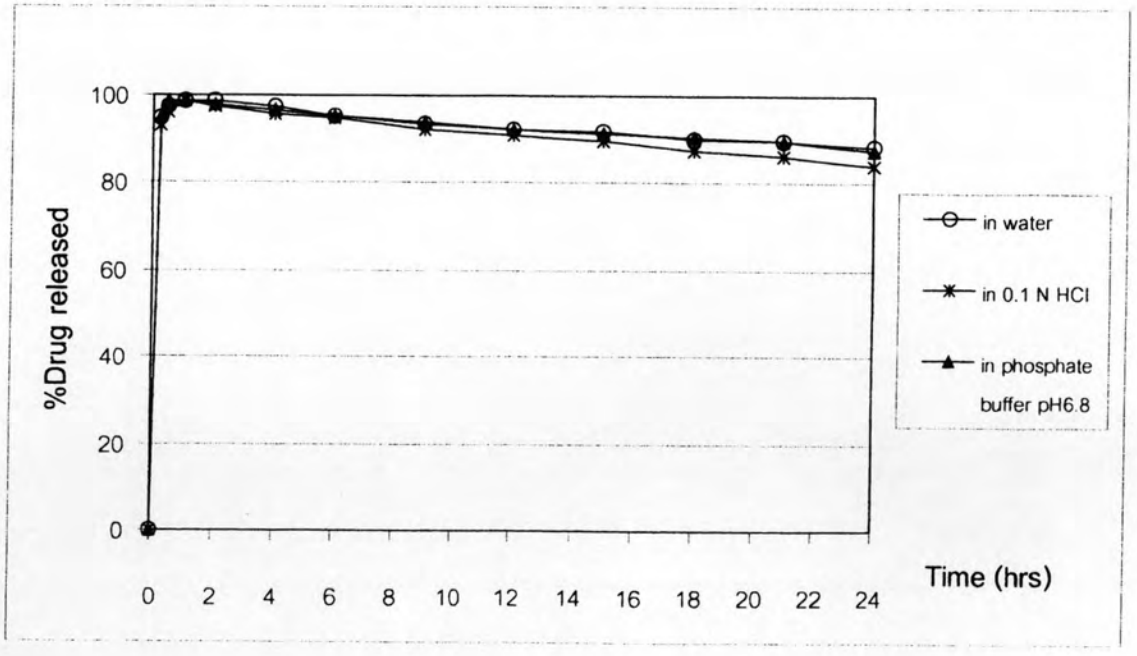


Figure 22 Dissolution profiles of Zerit® IR in different dissolution mediums

11.2 Dissolution Profiles of Film Coated stavudine pellets

11.2.1 Effect of Ethylcellulose Amount on the Released Profiles of Film Coated stavudine pellets

The release profile of coated stavudine pellets with the different percentages of ethylcellulose are shown in Figure 23. The release of stavudine from film coated pellets depended on percent of film coating level. The results showed that ethylcellulose film could retard the percentage release of stavudine from pellets. When the amount of ethylcellulose increased, the amount of stavudine released decreased. The fastest release rate was observed from the coated pellets with 5% of ethylcellulose and the slowest release rate was observed from the coated pellets with 20% of ethylcellulose. These because at high percentage coating level drug solution had to take time to diffuse through a thicker membrane before dissolving in the surrounding medium. This result corresponded with Sheen, et al (1992); Yuen, Deshmukh and Newton (1993); Chetty and Dangor (1994); Sadeghi, et al (2001). However, there are some researchers studied pharmacokinetic of stavudine in human and reported that stavudine had very short half life about 2-3 hours. Therefore, to maintain plasma drug concentration in vivo study covering 24 hours, in this study the dissolution profile was expected to prolong percentage of drug release about 80% at 18 hours. However, the result from dissolution test indicated that using ethylcellulose alone in coating formulation could not modify the release of stavudine to meet the requirement. Therefore, combination of ethylcellulose and HPMC at various proportions in each coating level was required.

11.2.2 Effect of Ratio of HPMC to Ethylcellulose on the Released Profiles of Film Coated stavudine pellets

The release profile of coated stavudine pellets with the different percentages of ethylcellulose are shown in Figure 24 to 29. The release of stavudine through the ethylcellulose film containing various concentration of HPMC depended on ratio of HPMC and ethylcellulose in film coating layer. The results from dissolution test indicated that increasing HPMC content in the coats increased the amount of drug released. Therefore, the ratio of HPMC to ethylcellulose in coating layer had a major effect on the release rate of drug. A lag time was observed before establishing the controlled-release

profile. The duration of lag time increased with increasing coating level, but decreased as the amount of HPMC in the coats increased. This result was similar to report by Sadeghi, et al (2001).

Hydroxypropyl methylcellulose is a water-soluble polymer and in this study it was used as a film modifier on dissolution profile of coated stavudine pellets. It could be explained that when pellets were immersed in the dissolution medium, soluble part of the film (HPMC) would dissolve and leach out, leaving pore or channels for drug release. In this case, drug could be dissolved and passed through pores to the dissolution medium. The more HPMC content was, faster the release rate was. These results were similar to report by some researchers such as Sheen, et al. (1992) found that pellets coated with combination of Surelease® and HPMC had faster release rate than those coated with Surelease® alone. Yuan, Deshmukh and Newton (1993) found that pellets coated with ethylcellulose containing coating additives (such as acacia, NaCl, Methylcellulose, PEG400 and PEG4000) increased drug release rate but the rates depended on additive properties. Chetty and Dangor (1994) found that release rate was increased with increasing HPLC level in ethylcellulose film. However, these results contradict those published by Donbrow and Samuelov (1980) for the effect of HPC as an additive in ethylcellulose film. These authors stated that the increase in permeation through the ethylcellulose/HPC film was due to retention of the HPC in the film and the formation of swollen hydrated channels. The differences in the type of cellulose ethers used. The reduced interaction of HPMC with ethylcellulose due to its increased substitution (Sakellariou et al., 1986) may be responsible for the leaching of HPMC from these films.

From the dissolution profile, it was observed that coated stavudine pellets formulation S20H5 could prolong percentage of drug release about 80% at 18 hours in pH change system. Therefore, this formulation was interesting and selected to study in vivo. However, it is noticed that after 24 hours of dissolution test stavudine in this formulation was not completely released. The pellets which remained in the dissolution vessels were collected and analysed for drug content. It was found that there were unreleased drug in pellets. The reason might due to core pellets had high content of microcrystalline cellulose and so that the drug might remained intact with microcrystalline cellulose during dissolution test. This result is agreed with Millili and Schwartz (1990). They found that drug release from microcrystalline cellulose pellets using water as granulating liquid remained intact during at least 12 hours of dissolution test.

11.2.3 Morphology and Dissolution Evaluation of stavudine released from selected formulation (Formulation S20H5)

The result from scanning electron microscope were shown in Figure 30. The result showed that stavudine pellets coated with ethylcellulose at 20%w/w of coating level containing 5% HPMC before dissolving, had round shape with smooth and consistent surface. After dissolution test, film coated pellets still had round shape, but the coating layers did not adhere to the surface core. Some pores on the film coating layer and channels were found in the cross-section view of film coating layer. Interestingly, the cracks were not found on the surface of coated stavudine pellets. These appearances indicated that the mechanism of drug release is controlled by polymer film as drug released through pores or channels during dissolution test.

Table 27 summarize the values of release rate constant (*K*), correlation coefficient (*r*) and lag time obtained from dissolution data corresponding to 10%-75% release of stavudine from coated pellets formulation S20H5 in various dissolution media. No results for drug released in 0.1 N HCl were reported due to amount of drug released in 0.1 N HCl were low and data points were insufficient for regression analysis.

The correlation coefficients of stavudine released in various dissolution media were close to 1 and all dissolution data were fitted to the equation of $Q = K t$, where Q is the percentage of drug released at time t and *K* is zero-order release rate constant. Therefore, the result revealed that the zero-order kinetic model could be the most applicable model to all the release data.

The zero order release rate of stavudine from coated pellets formulation S20H5 were between 3.2872-5.7546 $\%hour^{-1}$ and the range order of release rate of stavudine released were as follow as in ; pH change system > phosphate buffer pH 6.8 > water, respectively.

Lag times of drug released from coated pellets formulation S20H5 were between 0.1065-1.7612 hours and the range orders of lag time of stavudine released were as follow as in; pH change system > phosphate buffer pH 6.8 > water, respectively.

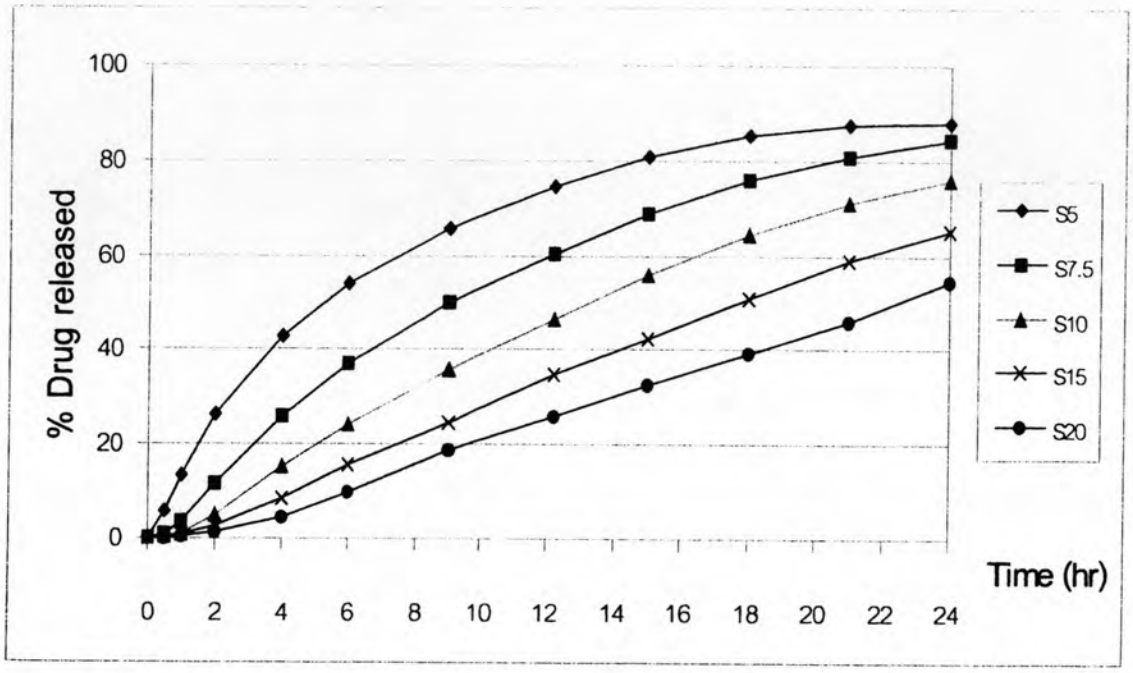


Figure 23 Dissolution profiles of stavudine pellets coated with Surelease® in water

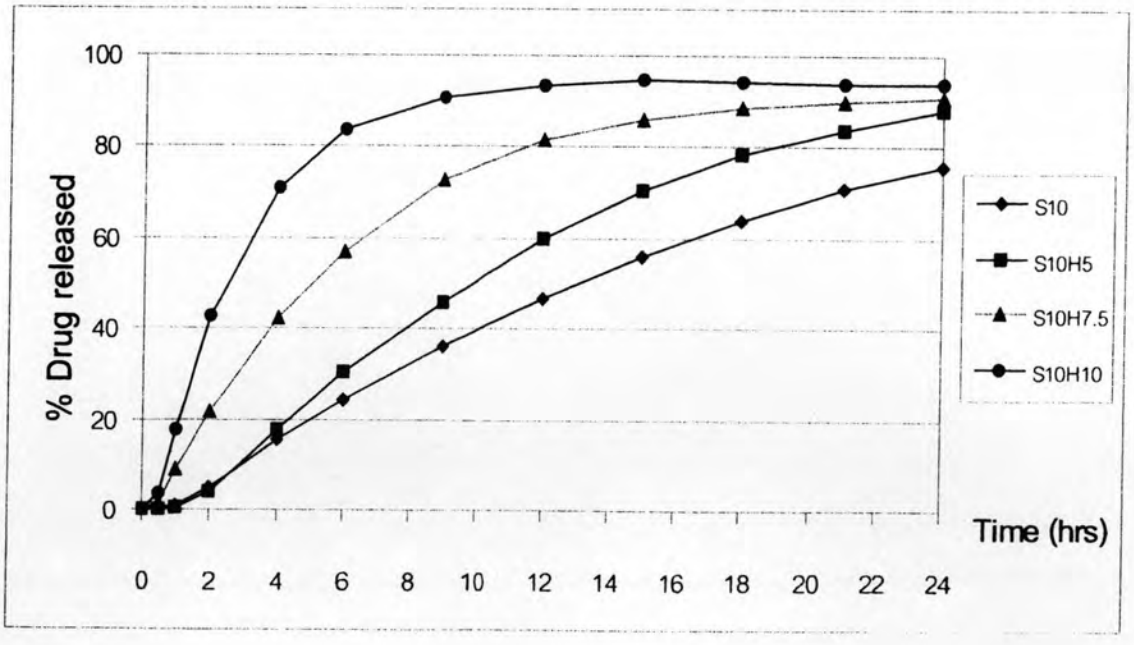


Figure 24 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV at 10% weigh gain in water

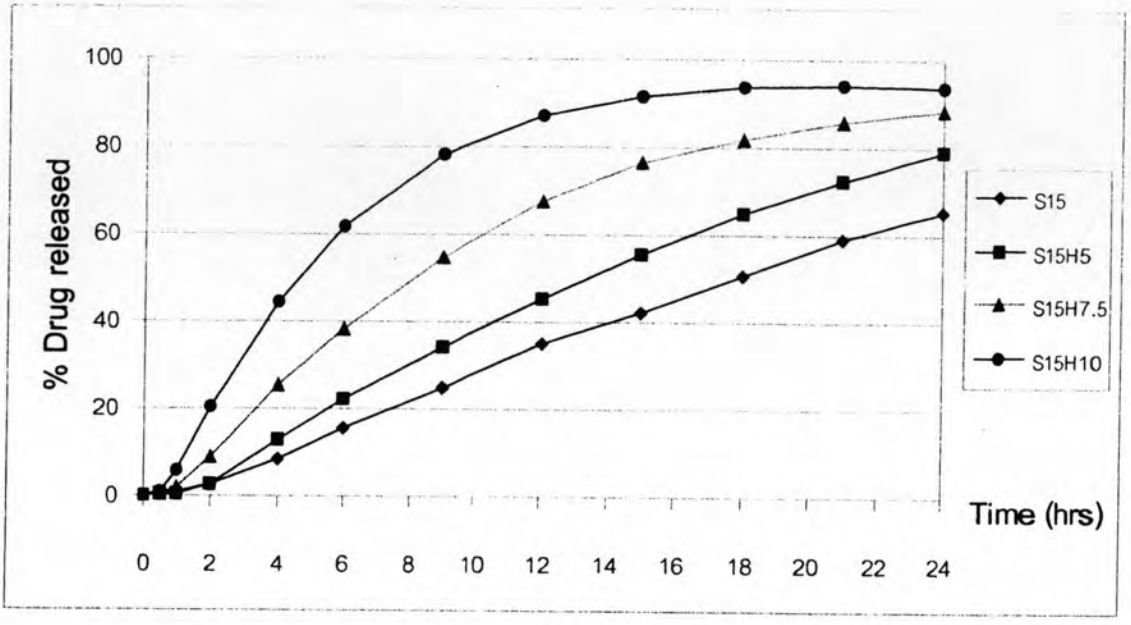


Figure 25 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV at 15% weigh gain in water

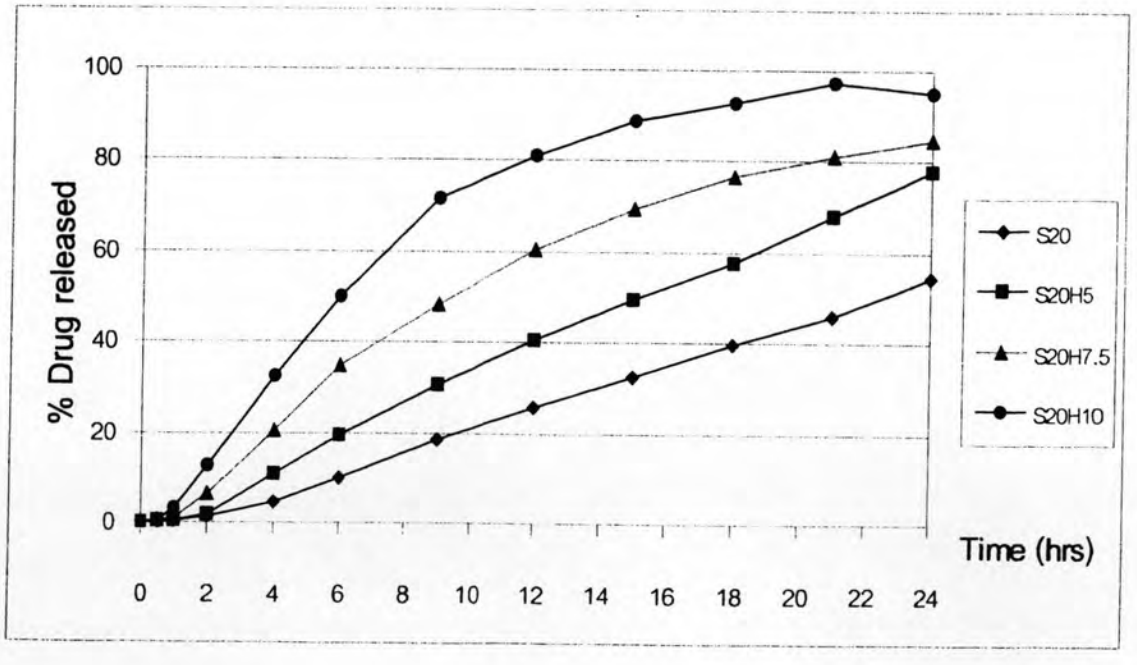


Figure 26 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV at 20% weigh gain in water

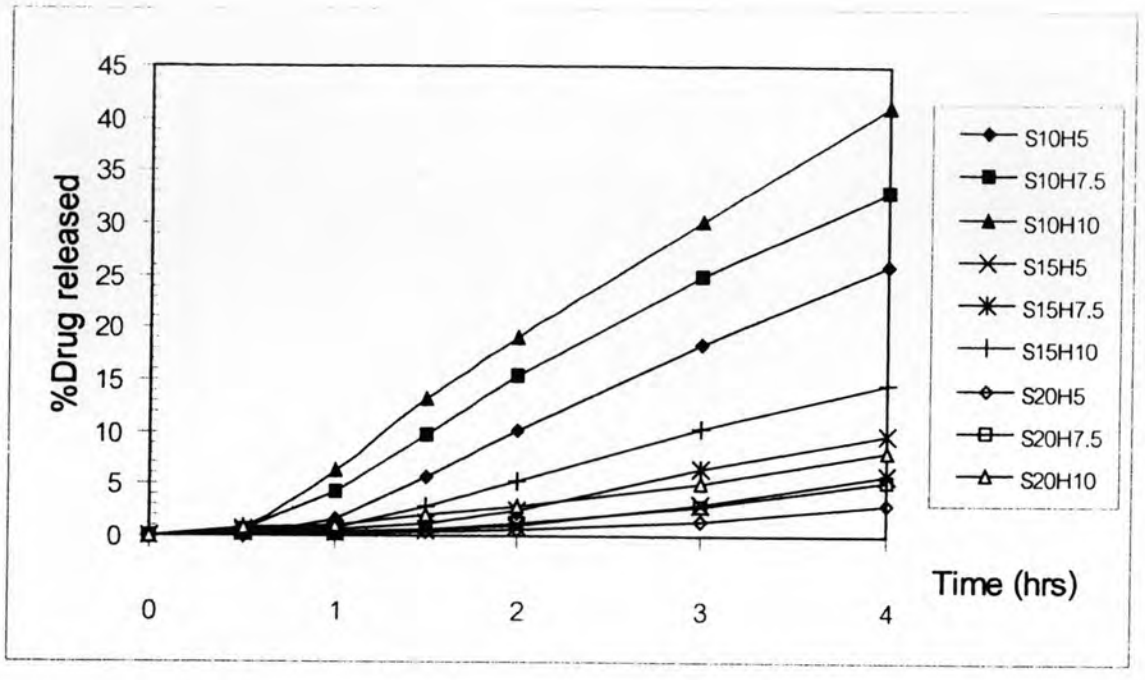


Figure 27 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV in 0.1 N HCl

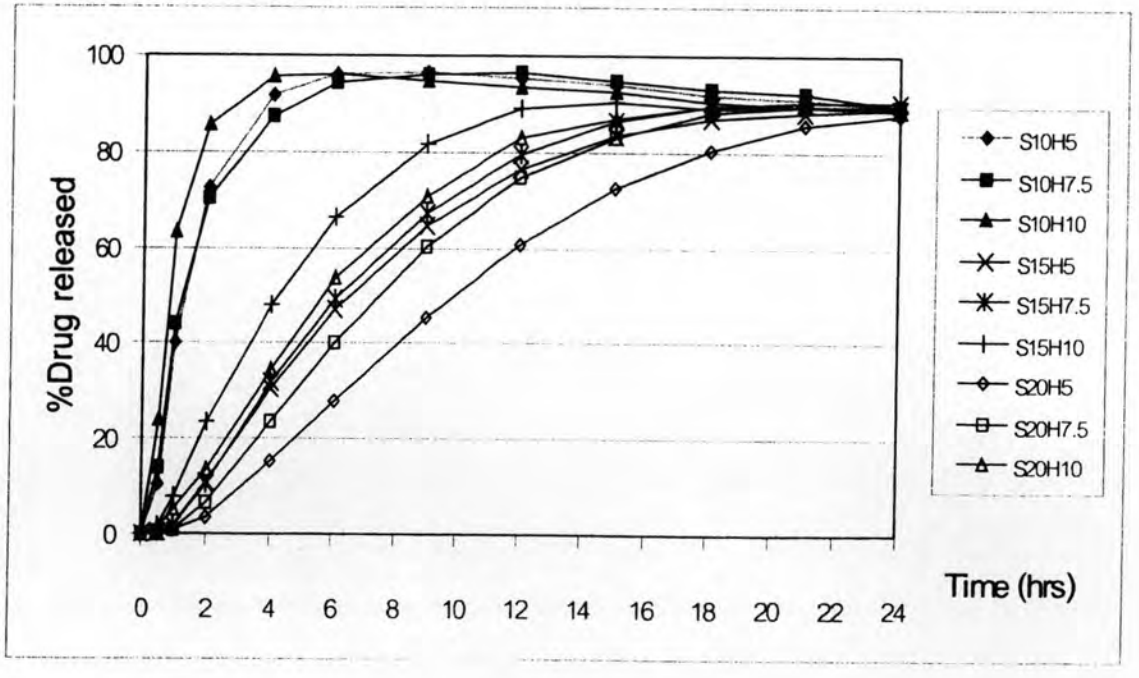


Figure 28 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV in phosphate buffer pH 6.8

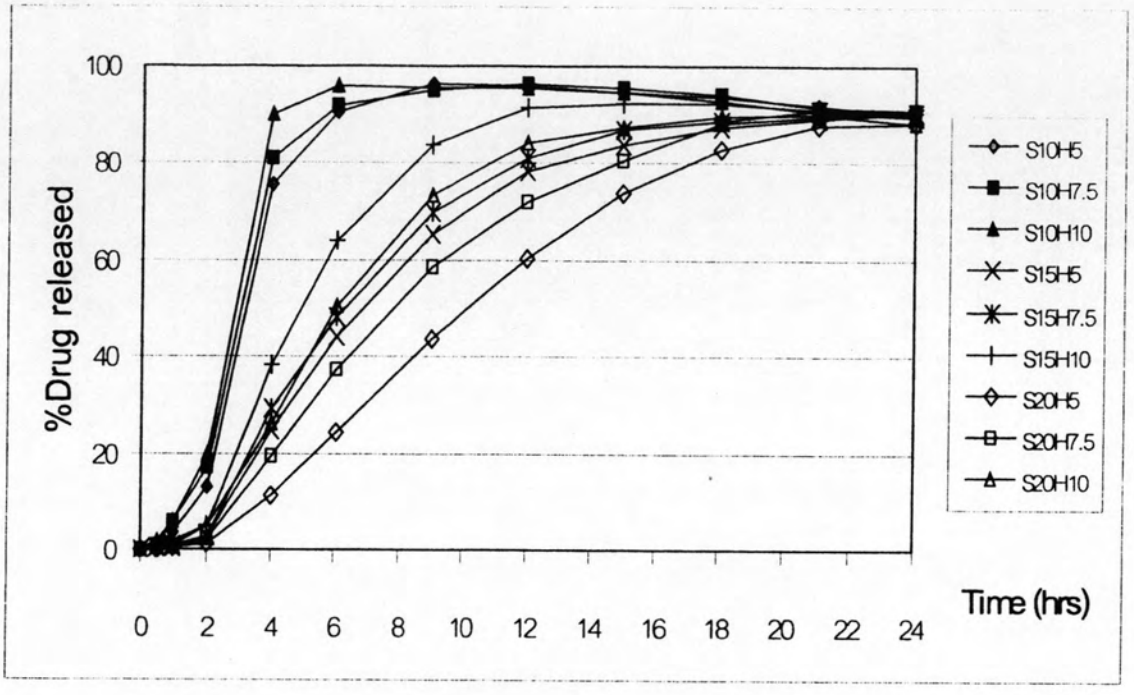


Figure 29 Dissolution profiles of stavudine pellets coated with Surelease® containing HPMC E15 LV in pH change system

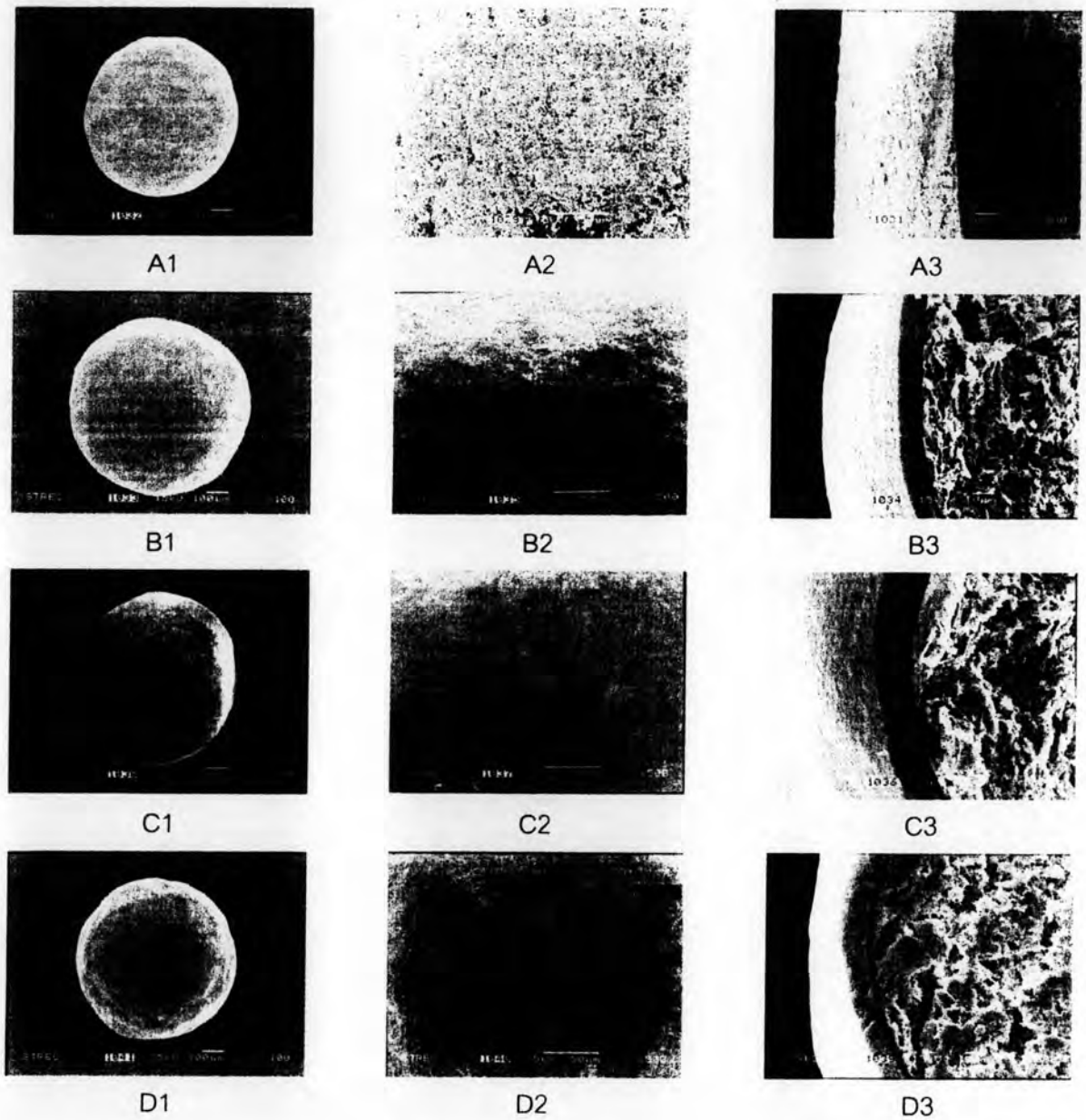


Figure 30 Photomicrographs of stavudine pellets (Formulation S20H5) before and after dissolution test

(A1, A2, A3 are pellets before dissolution test

at ×100, ×500, ×1000 (cross-section) of magnification

B1, B2, B3 are pellets after dissolution test in water

at ×100, ×500, ×1000 (cross-section) of magnification)

C1, C2, C3 are pellets after dissolution test in 0.1 N HCl

at ×100, ×500, ×1000 (cross-section) of magnification)

D1, D2, D3 are pellets after dissolution test in phosphate buffer pH 6.8

at ×100, ×500, ×1000 (cross-section) of magnification)

Table 27 Value of release rate constant, Correlation coefficient and Lag times obtained from dissolution profile of coated stavudine pellets formulation S20H5 in various dissolution mediums

	Dissolution medium		
	Water	phosphate buffer pH 6.8	pH change system
Zero order			
K (%.hr^{-1})	3.2872	5.2038	5.7546
R^2	0.9962	0.9995	0.9989
Lag times (t_{lag}) (hr)	0.1065	0.6752	1.7612
First Order			
K (hr^{-1})	0.6740	0.9611	0.9876
R^2	0.9610	0.8153	0.9503
Lag times (t_{lag}) (hr)	-	-	-
Higuchi's model			
K (%. $hr^{-0.5}$)	21.7621	30.7526	33.9718
R^2	0.9959	0.9927	0.9942
Lag times (t_{lag}) (hr)	1.5550	1.5147	1.6971

12. Stability result

12.1 Assay for Content of active ingredient and thymine degradation compound

Stavudine pellets which kept in the polyethylene bottles at initial time and at stability period were assayed for content of active ingredient and thymine degradation compound. The results are present in Table 28 and Figure 31 to 32. It indicated that thymine degradation compound was increased when product were kept for long time. As shown that thymine degradation compound in stavudine pellets at six months was higher than of at initial time. The content of stavudine in stavudine pellets was within the rage of 90-110 percent of label amount and thymine degradation compound was not more than 3%w/w all along the stability test. The stability test indicated that this product could have the shelf life for 2 years at room temperature in this container.

Table 28 Content of stavudine and thymine detected from stavudine pellets stored at 30 ^{0}C, 65 %RH and 40 ^{0}C, 75 %RH in the polyethylene bottles

Storage period	30 ^{0}C, 65%RH		40 ^{0}C, 75%RH	
	Stavudine* (%L.A.)	Thymine* (%w/w)	Stavudine* (%L.A.)	Thymine* (%w/w)
0 Month	99.58 (1.29)	0.26 (0.01)	99.35 (2.06)	0.32 (0.01)
1 Month	99.74 (2.10)	0.39 (0.02)	98.27 (1.79)	0.57 (0.02)
2 Months	99.08 (1.67)	0.58 (0.01)	98.56 (2.13)	0.95 (0.02)
3 Months	98.69 (1.58)	0.84 (0.01)	97.41 (1.35)	1.49 (0.01)
4 Months	99.06 (2.32)	0.62 (0.02)	96.08 (1.98)	2.08 (0.02)
5 Months	97.63 (1.34)	1.35 (0.01)	96.85 (1.76)	2.57 (0.03)
6 Months	98.45 (1.41)	1.18 (0.02)	95.73 (2.45)	2.84 (0.03)

*The results are averaged from triplicate determinations

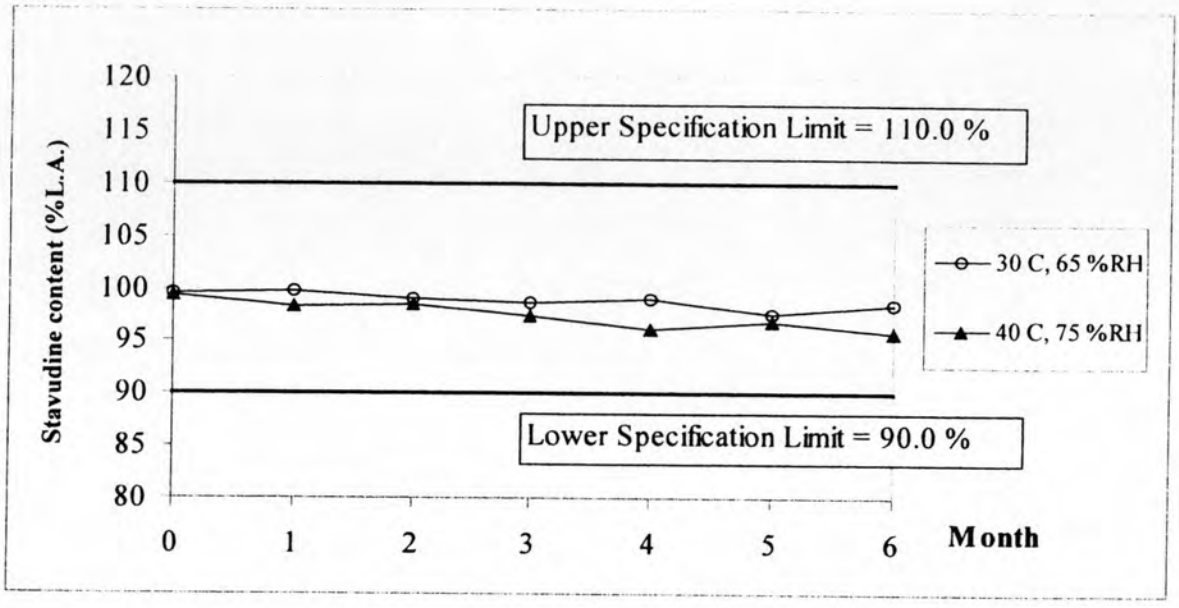


Figure 31 Content of stavudine detected from stavudine pellets kept at 30 ^{0}C, 65 %RH and 40 ^{0}C, 75 %RH in the polyethylene bottles

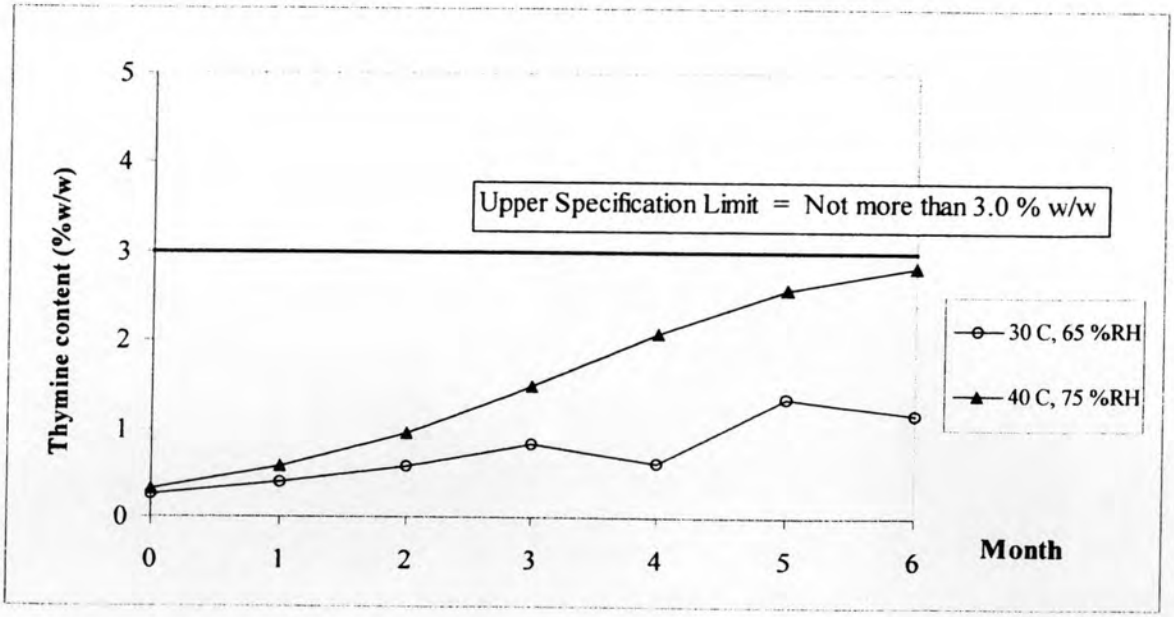


Figure 32 Content of thymine detected from stavudine pellets kept at 30 ^{0}C, 65 %RH and 40 ^{0}C, 75 %RH in the polyethylene bottles

12.2 Stability dissolution test

The dissolution profiles of stavudine pellets at initial time was compared with stavudine pellets at stability period using difference factor (f_1) and similarity factor (f_2). The stability dissolution data are shown in Appendix D.

When products were kept at 30 ^{0}C, 65 %RH for 6 months, the stability dissolution profiles of stavudine pellets in water, 0.1 N HCl, phosphate buffer pH 6.8 and pH-change system were shown in Figure 33 to 36. Results in Table 29 to 32 demonstrated that the different factor (f_1) and similarity factor (f_2) between stavudine pellets at initial time and stavudine pellets at stability period in four dissolution medium system met the acceptance criteria (f_1 = 0-15 and f_2 = 50-100). These results indicated that dissolution profiles of stavudine pellets at stability period was equivalent to that of stavudine pellets at initial time and no change of product release in water, 0.1 N HCl, phosphate buffer pH 6.8 and pH-change system during 6 months of stability state 30 ^{0}C, 65 %RH.

When products were kept at 40 ^{0}C, 75 %RH for 6 months, the stability dissolution profiles of stavudine pellets in water, 0.1 N HCl, phosphate buffer pH 6.8 and pH-change system were shown in Figure 37 to 40. Results in Table 33 to 36 demonstrated that the different factor and similarity factor between stavudine pellets at initial time and stavudine pellets at stability period in three dissolution medium system met the acceptance criteria as like when products were kept at 30 ^{0}C, 65 %RH. These results indicated that dissolution profiles of stavudine pellets at stability period was equivalent to that of stavudine pellets at initial time and no change of product released in water, 0.1 N HCl, phosphate buffer pH 6.8 and pH-change system during 6 months of stability state 40 ^{0}C, 75 %RH.

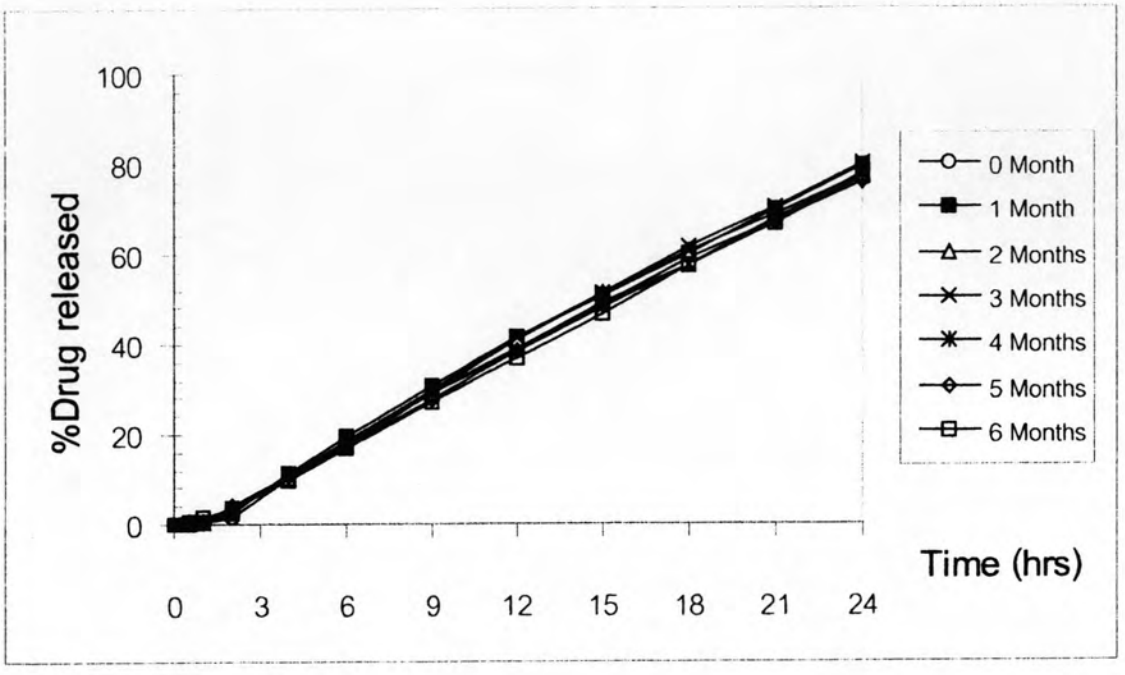


Figure 33 Dissolution Profiles of stavudine pellets kept at 30 ^{0}C, 65 %RH in water

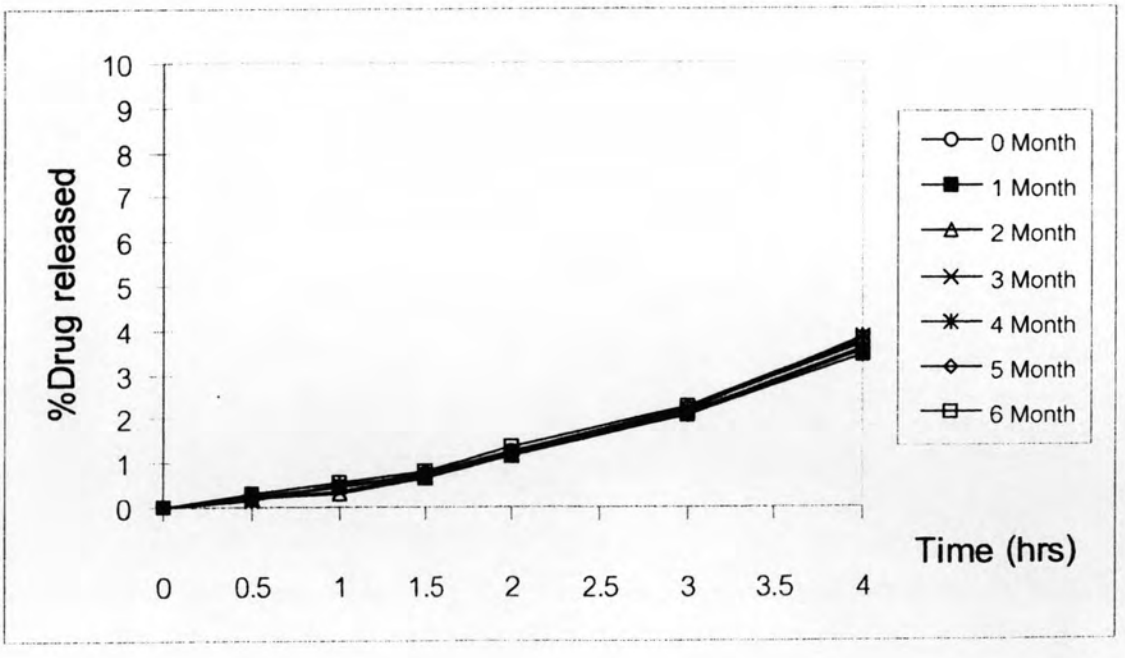


Figure 34 Dissolution Profiles of stavudine pellets kept at 30 ^{0}C, 65 %RH in 0.1 N HCl

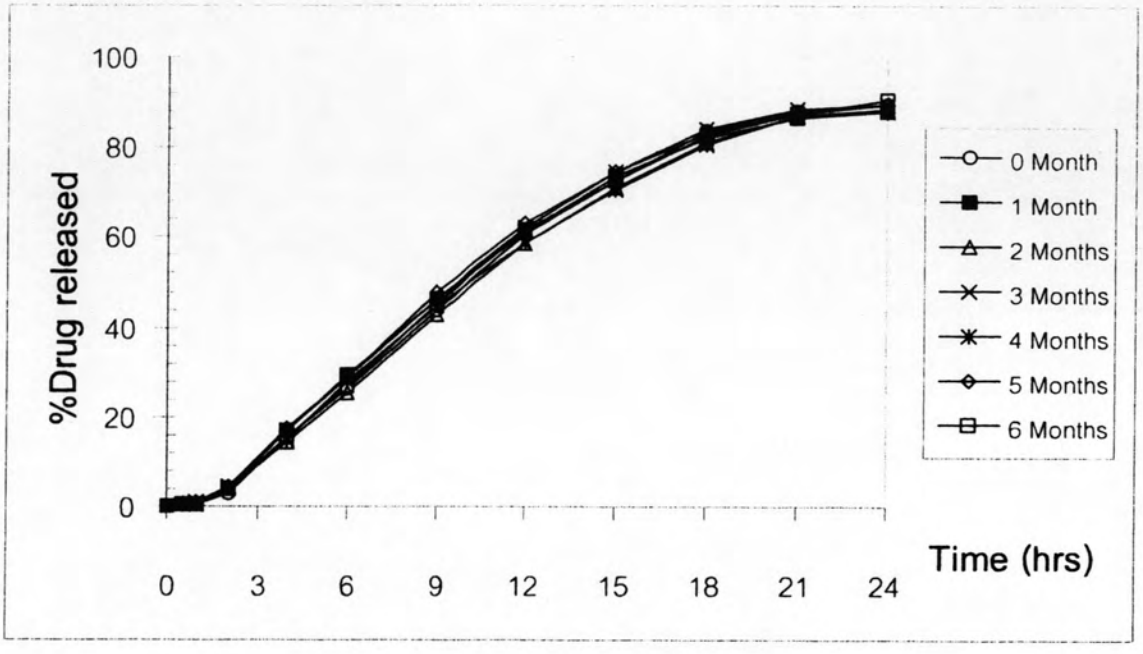


Figure 35 Dissolution Profiles of stavudine pellets kept at 30 ^{0}C, 65 %RH in phosphate buffer pH 6.8

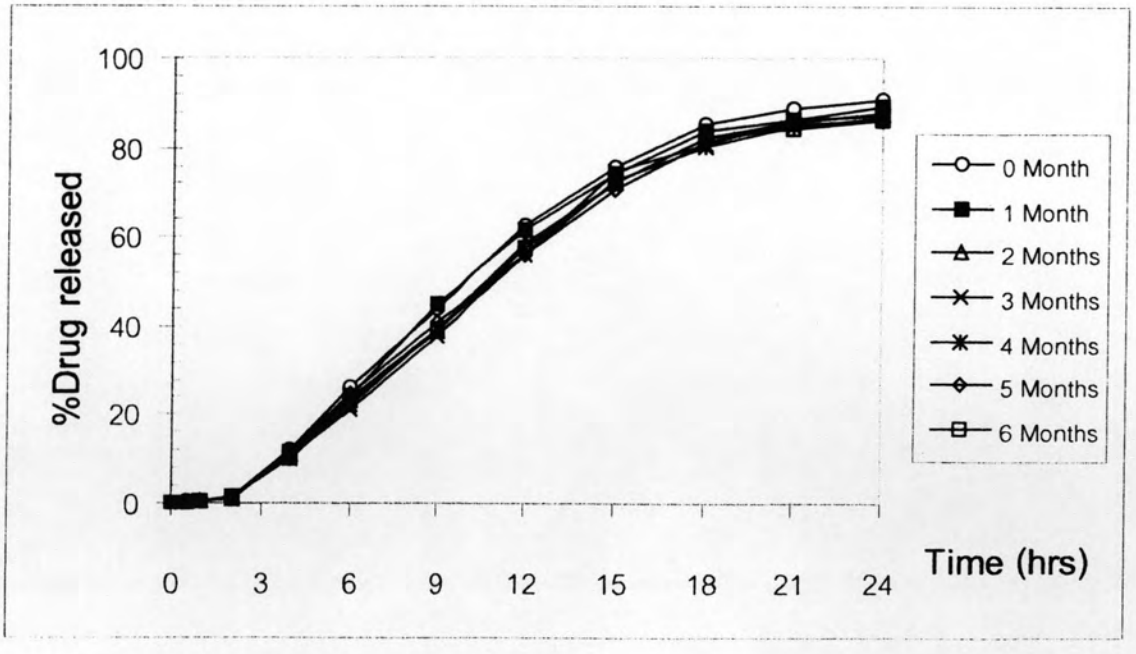


Figure 36 Dissolution Profiles of stavudine pellets kept at 30 ^{0}C, 65 %RH in pH-change system

Table 29 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in water of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (30 ^{0}C, 65 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	4.43	86.69
0 month and 2 month	3.26	88.83
0 month and 3 month	5.28	83.07
0 month and 4 month	1.38	96.16
0 month and 5 month	2.75	90.06
0 month and 6 month	3.47	89.09

Table 30 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in 0.1 N HCl of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (30 ^{0}C, 65 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	6.03	99.89
0 month and 2 month	6.96	99.89
0 month and 3 month	4.52	100.00
0 month and 4 month	5.91	99.89
0 month and 5 month	4.76	99.89
0 month and 6 month	5.57	99.89

Table 31 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in buffer pH 6.8 of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (30 ^{0}C, 65 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	2.87	87.47
0 month and 2 month	2.48	90.18
0 month and 3 month	2.74	87.79
0 month and 4 month	2.86	87.58
0 month and 5 month	2.02	92.37
0 month and 6 month	1.54	94.50

Table 32 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in pH change system of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (30 ^{0}C, 65 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	2.70	88.14
0 month and 2 month	5.57	75.71
0 month and 3 month	6.37	71.86
0 month and 4 month	6.96	71.32
0 month and 5 month	6.04	73.21
0 month and 6 month	6.28	73.50

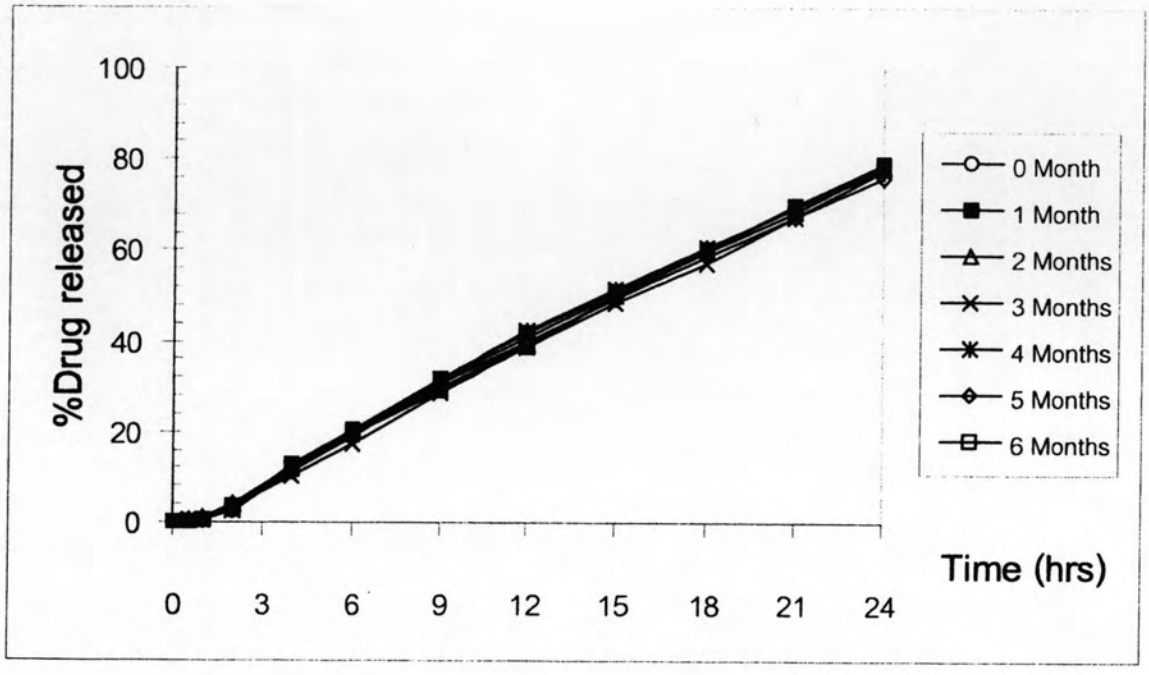


Figure 37 Dissolution Profiles of stavudine pellets kept at 40 ^{0}C, 75%RH in water

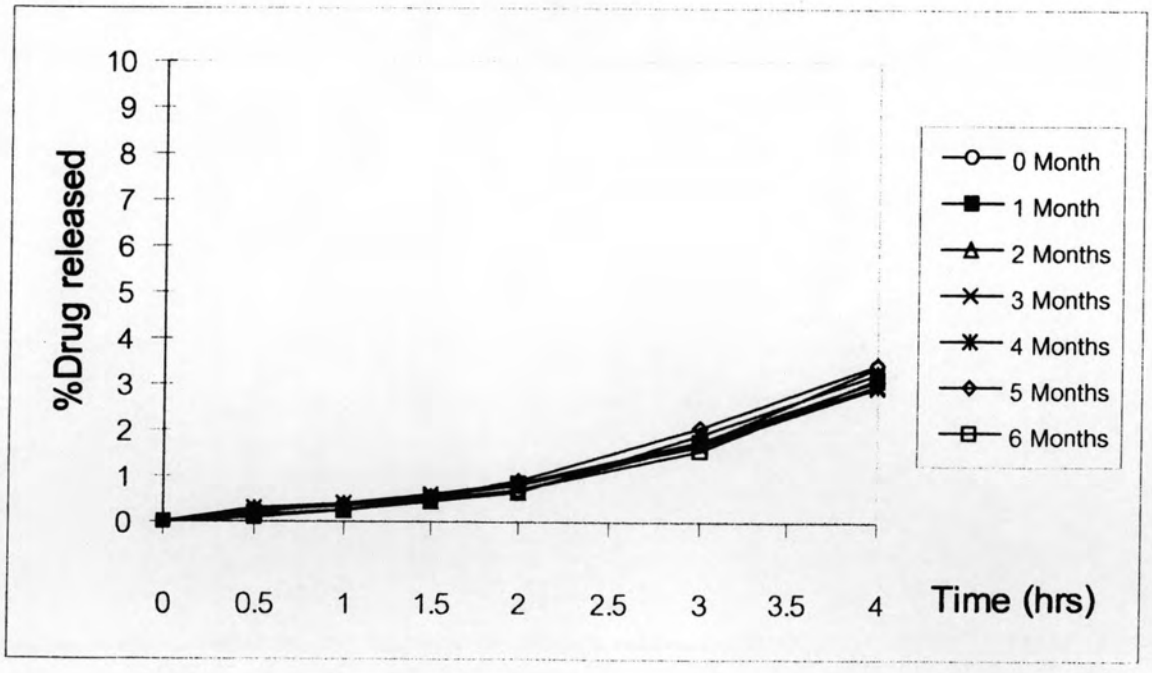


Figure 38 Dissolution Profiles of stavudine pellets kept at 40 ^{0}C, 75%RH in 0.1 N HCl

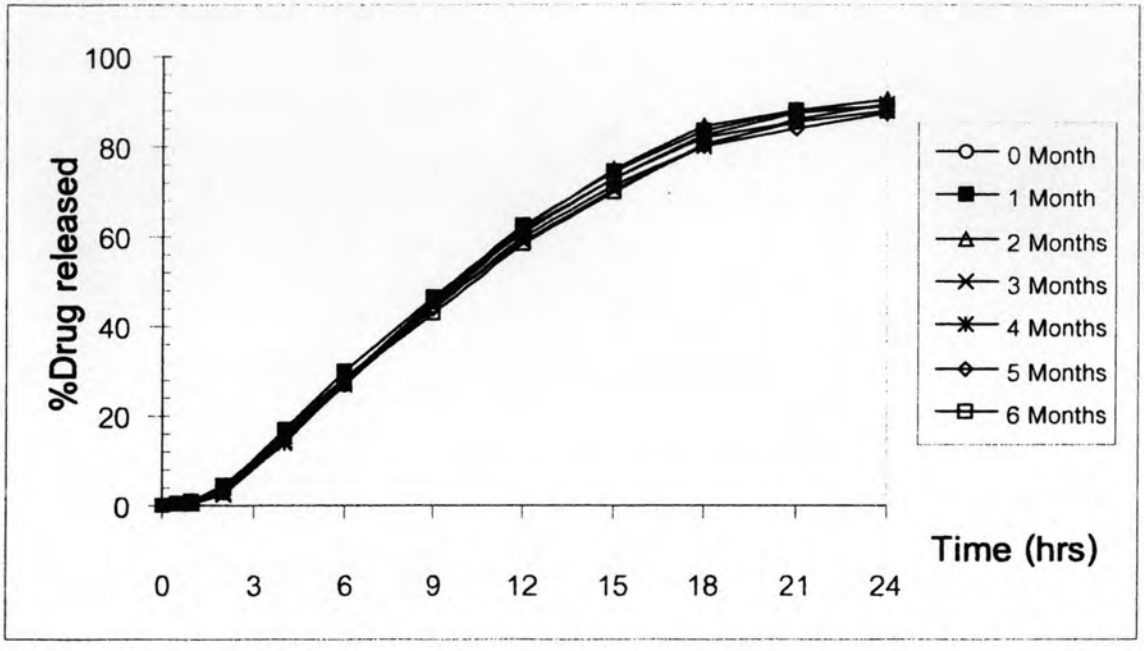


Figure 39 Dissolution Profiles of stavudine pellets kept at 40 ^{0}C, 75%RH in phosphate buffer pH 6.8

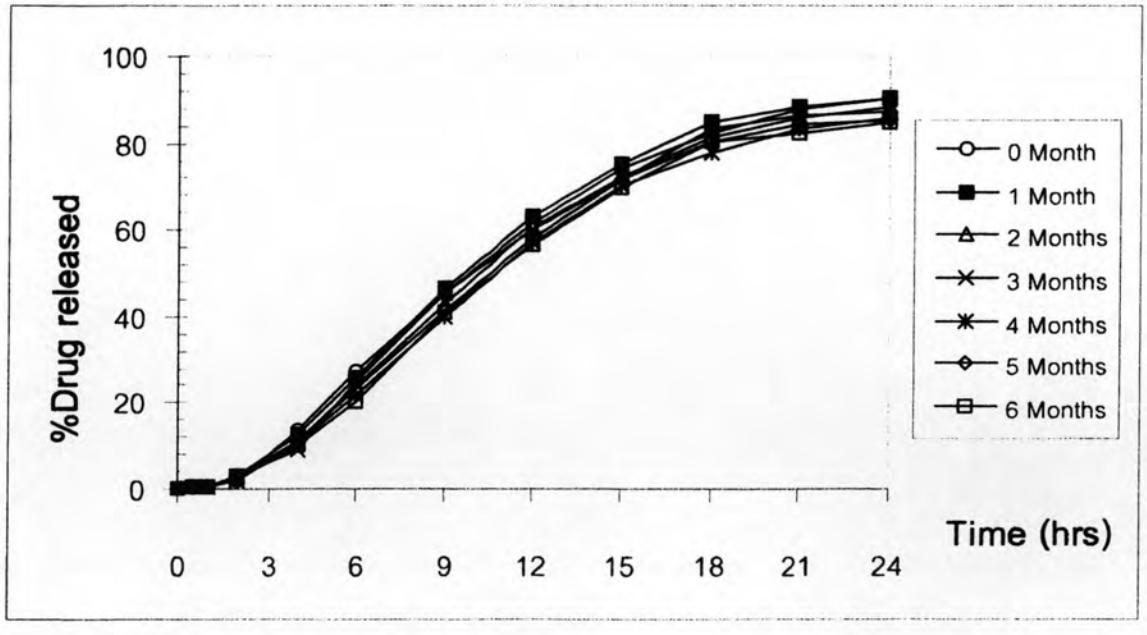


Figure 40 Dissolution Profiles of stavudine pellets kept at 40 ^{0}C, 75%RH in pH-change system

Table 33 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in water of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (40 ^{0}C, 75 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	1.37	96.36
0 month and 2 month	2.73	92.47
0 month and 3 month	4.71	84.52
0 month and 4 month	2.05	94.55
0 month and 5 month	2.80	89.50
0 month and 6 month	2.10	92.92

Table 34 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in water of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (40 ^{0}C, 75 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	10.45	99.78
0 month and 2 month	9.14	99.89
0 month and 3 month	12.33	99.68
0 month and 4 month	10.74	99.68
0 month and 5 month	10.16	99.68
0 month and 6 month	10.16	99.78

Table 35 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in buffer pH 6.8 of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (40 ^{0}C, 75 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	2.30	89.30
0 month and 2 month	2.01	92.26
0 month and 3 month	2.43	88.37
0 month and 4 month	1.45	94.70
0 month and 5 month	3.18	86.21
0 month and 6 month	3.19	85.85

Table 36 The Difference factor (f_1) and Similarity Factor (f_2) of various dissolution profiles in pH change system of stavudine pellets at stability period relative to stavudine pellets at initial time of storage condition (40 ^{0}C, 75 %RH)

stavudine pellets at various time	Difference factor (f_1)	Similarity Factor (f_2)
0 month and 1 month	2.05	91.34
0 month and 2 month	3.41	85.14
0 month and 3 month	3.68	82.46
0 month and 4 month	7.28	71.32
0 month and 5 month	5.50	76.57
0 month and 6 month	7.60	69.94

B. In vivo study

1. In-vivo Analytical Method Validation

1.1 Selectivity/Specificity

Chromatograms of blank plasma, plasma spiked with stavudine, plasma spiked with degradation product (thymine), plasma spiked with internal standard (zidovudine), plasma spiked with mixture of stavudine, thymine and zidovudine, are shown in Figures 41 to 42.

The mean retention times of thymine, stavudine and zidovudine were 3.8, 4.8 and 10.4 minutes, respectively. Peaks of drug and internal standard were well separated from other interfering peaks from six different blank plasma samples. Peak of degradation product was interfered from peaks in blank plasma but it was completely separated from peaks of drug and internal standard. Therefore, in this study no effect of thymine to the presence of stavudine and zidovudine.

1.2 Linearity and standard calibration curve

The linearity of the assay procedure was demonstrated between stavudine concentrations of 50-2000 ng/ml. Five standard calibration curves of peak area ratios of stavudine to zidovudine versus concentrations are illustrated in Tables 37 to 41 and Figure 43.The linear regression of five calibration curves showed the typical coefficient of determination (r^2) of 0.9998, 0.9997, 0.9995, 0.9993 and 0.9992. Percent recovery of stavudine each concentration was within $\pm$15% except at the lowest stavudine concentration 50 ng/ml of calibration curve No.1, which the percent recovery was 83.28% and it was within $\pm$ 20%.

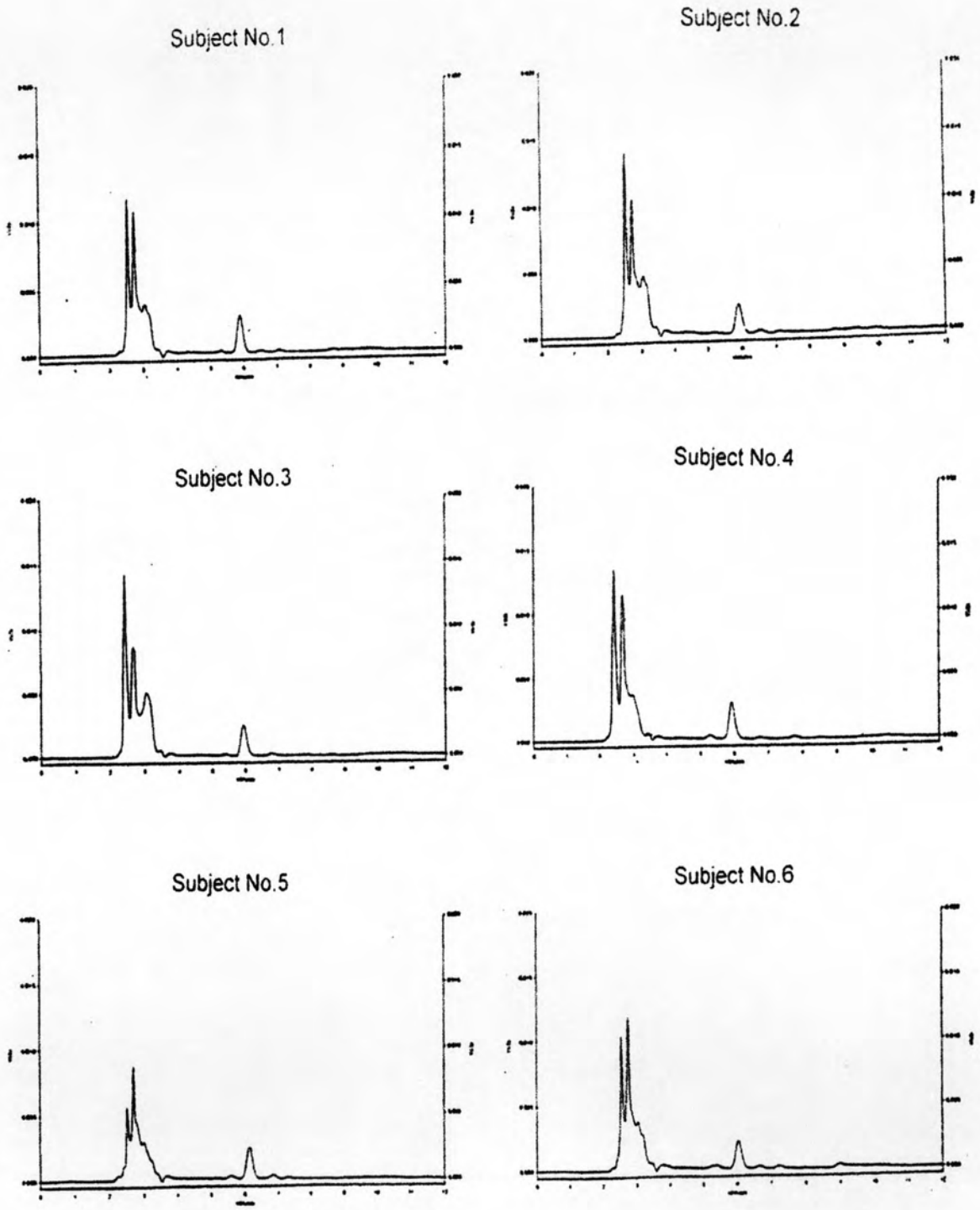


Figure 41 HPLC Chromatograms of Blank Plasma from Six Subjects (Subject No.1-6)

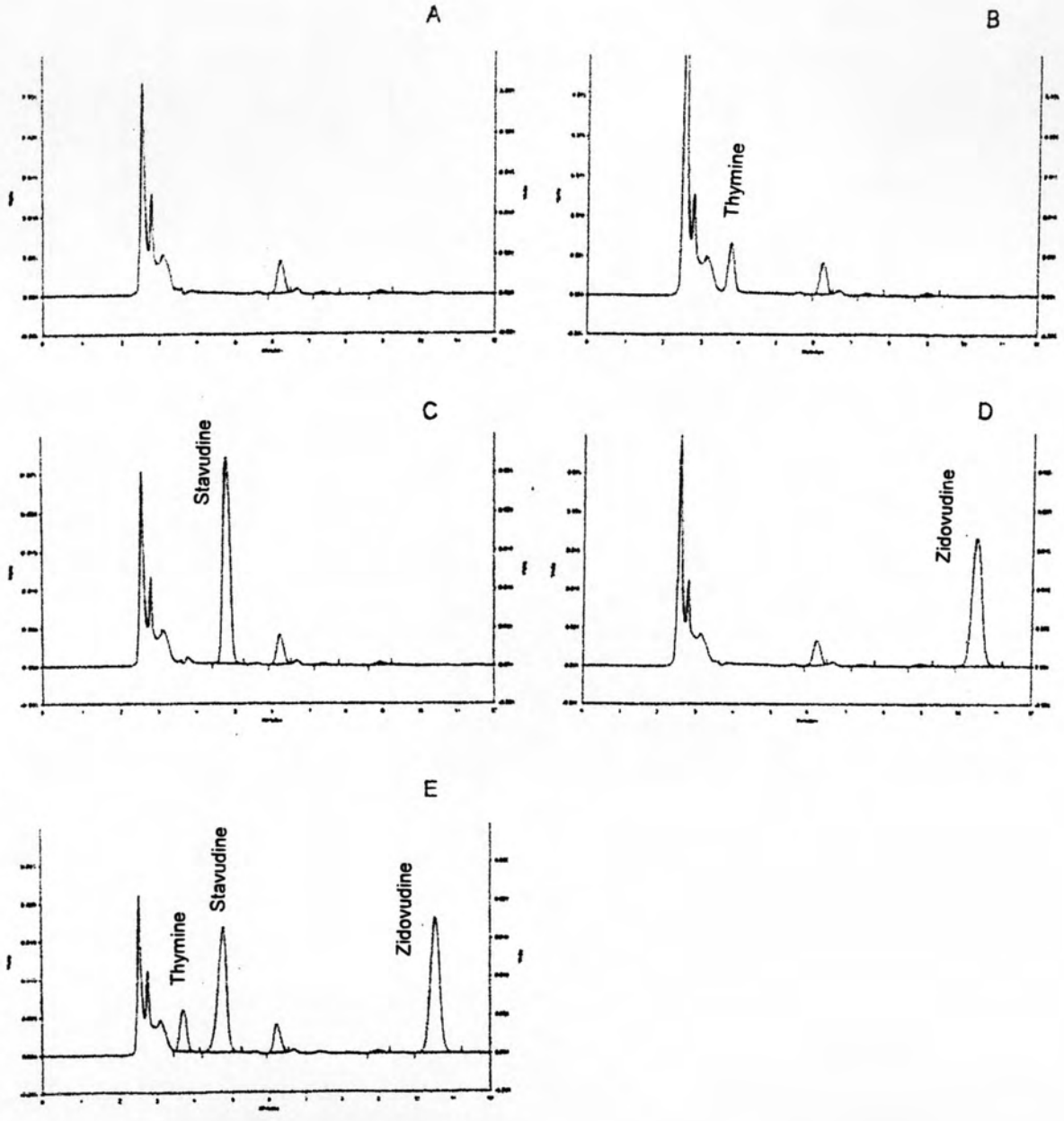


Figure 42 HPLC Chromatograms of

A. Blank Plasma

B. Plasma Spiked with Thymine

C. Plasma Spiked with Stavudine

D. Plasma Spiked with Zidovudine (Internal Standard)

E. Plasma Spiked with Thymine, Stavudine and Zidovudine

Table 37 Linearity of Curve No. 1 of HPLC Analytical Method for Determination of Stavudine in Plasma

Standard no.	Known Concentration (ng/ml)	Peak area Ratio	Estimated Concentration (ng/ml)	% recovery
1	50	0.025	41.640	83.28
2	100	0.0491	88.040	88.04
3	250	0.1392	268.240	107.30
4	500	0.2587	507.240	101.45
5	1000	0.5043	998.440	99.84
6	1500	0.7502	1490.240	99.35
7	2000	1.0059	2001.640	100.08

Where ; $r^2 = 0.9998$ $y = 0.00050x + 0.00508$

$$\text{Estimated concentration} = \frac{[\text{Peak area ratio} - 0.00508]}{0.00050}$$

$$\text{\% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

Table 38 Linearity of Curve No. 2 of HPLC Analytical Method for Determination of Stavudine in Plasma

Standard no.	Known Concentration (ng/ml)	Peak area Ratio	Estimated Concentration (ng/ml)	% recovery
1	50	0.0261	48.078	96.16
2	100	0.0502	95.333	95.33
3	250	0.1373	266.118	106.45
4	500	0.2536	494.157	98.83
5	1000	0.5031	983.373	98.34
6	1500	0.7512	1469.843	97.99
7	2000	1.0223	2001.412	100.07

Where ; $r^2 = 0.9997$ $y = 0.00051x + 0.00158$

$$\text{Estimated concentration} = \frac{[\text{Peak area ratio} - 0.00158]}{0.00051}$$

$$\text{\% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

Table 39 Linearity of Curve No. 3 of HPLC Analytical Method for Determination of Stavudine in Plasma

Standard no.	Known Concentration (ng/ml)	Peak area Ratio	Estimated Concentration (ng/ml)	% recovery
1	50	0.0263	43.600	87.20
2	100	0.0486	88.200	88.20
3	250	0.1301	251.200	100.48
4	500	0.2579	506.800	101.36
5	1000	0.5029	996.800	99.68
6	1500	0.7654	1521.800	101.45
7	2000	0.9878	1966.600	98.33

Where ; $r^2 = 0.9995$ $y = 0.00050x + 0.00450$

$$\text{Estimated concentration} = \frac{[\text{Peak area ratio} - 0.00450]}{0.00050}$$

$$\text{\% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

Table 40 Linearity of Curve No. 4 of HPLC Analytical Method for Determination of Stavudine in Plasma

Standard no.	Known Concentration (ng/ml)	Peak area Ratio	Estimated Concentration (ng/ml)	% recovery
1	50	0.0336	50.525	101.02
2	100	0.0621	98.881	98.78
3	250	0.1632	270.237	107.99
4	500	0.2987	499.898	99.95
5	1000	0.5836	982.780	98.27
6	1500	0.8620	1454.644	96.96
7	2000	1.1941	2017.525	100.88

Where ; $r^2 = 0.9993$ $y = 0.00059x + 0.00376$

$$\text{Estimated concentration} = \frac{[\text{Peak area ratio} - 0.00376]}{0.00059}$$

$$\text{\% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

Table 41 Linearity of Curve No. 5 of HPLC Analytical Method for Determination of Stavudine in Plasma

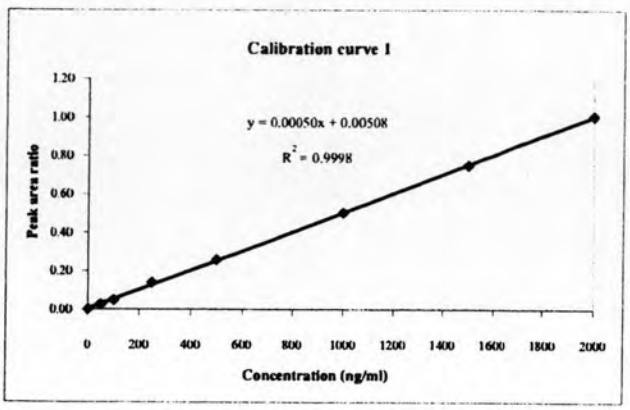
Standard no.	Known Concentration (ng/ml)	Peak area Ratio	Estimated Concentration (ng/ml)	% recovery
1	50	0.0336	48.542	96.99
2	100	0.0621	96.847	96.75
3	250	0.1632	268.203	107.18
4	500	0.2987	497.864	99.54
5	1000	0.5836	980.746	98.07
6	1500	0.8620	1452.610	96.83
7	2000	1.1941	2015.492	100.77

Where ; $r^2 = 0.9992$ $y = 0.00059x + 0.00496$

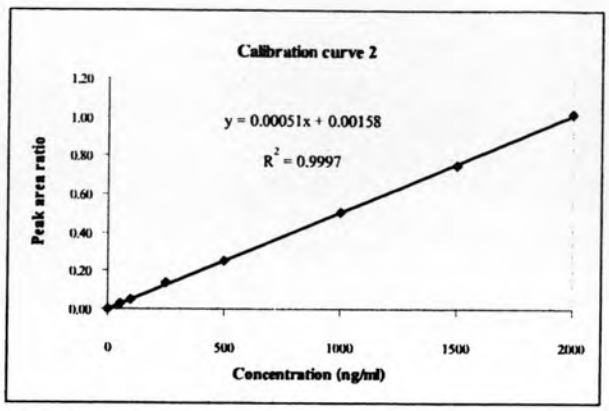
$$\text{Estimated concentration} = \frac{[\text{Peak area ratio} - 0.00496]}{0.00059}$$

$$\text{\% Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

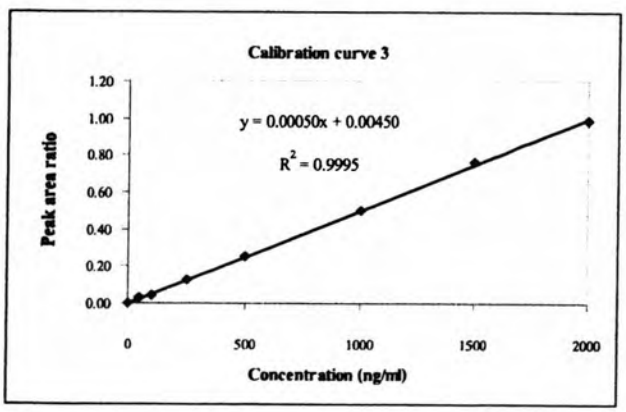
A.



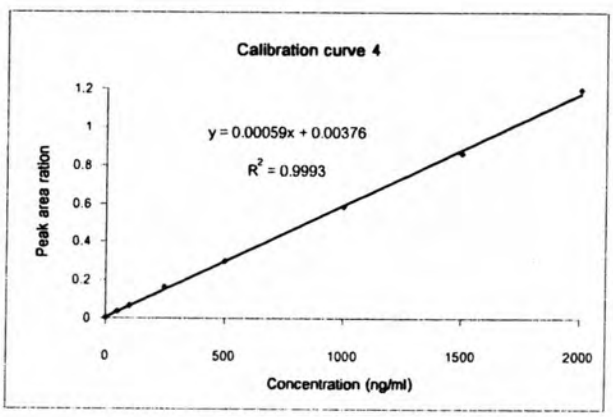
B.



C.



D.



E.

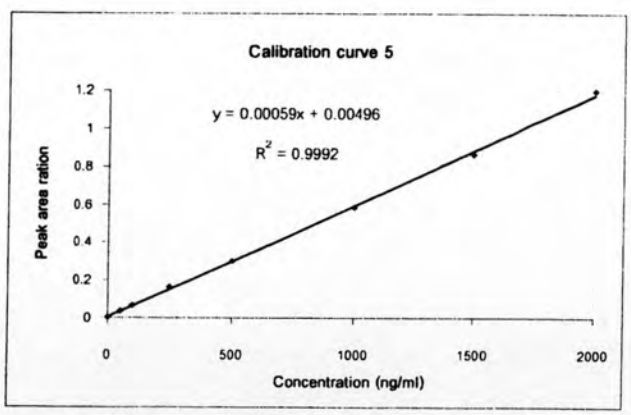


Figure 43 Linearity of Curve of Analytical Method for Determination of Stavudine in Plasma

A. Calibration Curve No.1 B. Calibration Curve No.2

C. Calibration Curve No.3 D. Calibration Curve No.4

E. Calibration Curve No.5

1.3 Lower Limit of Quantification (LLOQ)

The lower limit of quantification of the analysis method of stavudine was found to be 50 ng/ml. The accuracy of stavudine at 50 ng/ml was 92.97% with %C.V. of 8.00%. This result was accepted according to the fact that this level is the lowest concentration on the standard calibration curve and its concentration can be still determined with acceptable accuracy (%Recovery was range of ±20%) and precision was less than 20%. All data are presented in Table 42.

Table 42 Lower Limit of Quantification of HPLC Analytical Method for Determination of Stavudine in Plasma

Analysis no.	Known Concentration (ng/ml)	Estimated Concentration (ng/ml)	% Recovery
1	50.0	41.640	83.28
2	50.0	48.078	96.16
3	50.0	43.600	87.20
4	50.0	50.525	101.20
5	50.0	48.542	96.99
Mean		46.477	92.97
S.D.		3.70	7.44
%C.V.		7.97	8.00

1.4 Accuracy, Within-Run and Between-Run Precision

The accuracy, within- and between run precision of the analysis method for stavudine were assessed by analysing quality control samples spiked with known amount of stavudine. The results are shown in Tables 43 to 45. The results represent that percent recovery range of stavudine was 91.11 to 94.26%. Percent of coefficient variations for within- and between-run precision were 0.65-6.77% and 1.56-6.38%, respectively. These results were within acceptance criteria for accuracy (recovery ±15%) and precision (%C.V.<15%).

Table 43 Accuracy of HPLC Analytical Method for Determination of Stavudine in Plasma

Sample	QCL		QCM		QCH	
	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)
1	100	87.015	750	690.856	1750	1666.129
2	100	88.014	750	712.474	1750	1643.897
3	100	85.012	750	701.634	1750	1654.656
4	100	97.982	750	689.665	1750	1642.524
5	100	97.537	750	690.248	1750	1640.564
Mean	91.112		696.975		1649.554	
S.D.	6.17		9.98		10.76	
%C.V.	6.77		1.43		0.65	
% Recovery	91.11		92.93		94.26	

Table 44 Within-Run Precision of HPLC Analytical Method for Determination of Stavudine in Plasma

Sample	QCL		QCM		QCH	
	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)
1	100	87.015	750	690.856	1750	1666.129
2	100	88.014	750	712.474	1750	1643.897
3	100	85.012	750	701.634	1750	1654.656
4	100	97.982	750	689.665	1750	1642.524
5	100	97.537	750	690.248	1750	1640.564
Mean	91.112		696.975		1649.554	
S.D.	6.17		9.98		10.76	
%C.V.	6.77		1.43		0.65	

Table 45 Between-Run Precision of HPLC Analytical Method for Determination of Stavudine in Plasma

Sample	QCL		QCM		QCH	
	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)	Known Conc. (ng/ml)	Estimated Conc. (ng/ml)
1	100	85.321	750	686.324	1750	1644.943
2	100	87.656	750	681.877	1750	1651.626
3	100	93.559	750	661.762	1750	1702.811
4	100	97.832	750	667.665	1750	1642.260
5	100	98.414	750	661.605	1750	1643.018
Mean	92.556		671.847		1656.932	
S.D.	5.91		11.56		25.91	
%C.V.	6.38		1.72		1.56	

2. Stability studies

In order to determine the stability of stavudine in plasma, four studies were carried out: a short-term room temperature, a long-term, a freeze-thaw and processed samples stability studies.

2.1 Short-term room temperature stability

The short-term room temperature stability of stavudine in plasma data are presented in Table 46. The result showed that stavudine was tended to degrade after it was thawed at room temperature and kept at this temperature from 4 to 12 hours. The percent deviation from the zero time of low concentration and high concentration of stavudine were –5.69 to -15.34 % and –4.30 to -15.06 % , respectively. The results also obviously showed that after keeping plasma at room temperature for 12 hours, percent deviation from the zero time of both low concentration and high concentration of stavudine were higher than 15%. These results indicated that the samples should be rapidly extracted and analysed within 8 hours at room temperature.

2.2 Long-term stability

The long-term stability of stavudine in plasma data are presented in Table 47. The result showed that after keeping plasma at –20^{0}C for 8 weeks, the percent deviation from the zero time of low concentration and high concentration of stavudine were –2.58 to -3.67% and –1.42 to -3.55%, respectively. These results were within acceptance criteria (±15%) and indicated that stavudine samples were stable for 2 months when they were kept frozen at –20^{0}C. This storage time was sufficient for completion of drug analysis.

Table 46 Short-term Stability of HPLC Analytical Method for Determination of Stavudine in Plasma

Time (hour)	Known Concentration (ng/ml)	Estimated Concentration (ng/ml)	Mean Estimated Concentration (ng/ml)	S.D.	%Deviation
0	100	92.482 92.508 92.079	92.356	0.241	-
	1750	1656.224 1652.709 1655.686	1654.873	1.893	-
4	100	87.434 86.666 87.211	87.104	0.395	-5.69
	1750	1583.046 1583.745 1584.172	1583.654	0.568	-4.30
8	100	81.836 82.258 83.466	82.52	0.846	-10.65
	1750	1519.183 1520.148 1518.313	1519.215	0.918	-8.20
12	100	78.473 78.019 78.076	78.189	0.247	-15.34
	1750	1406.128 1405.493 1405.534	1405.718	0.355	-15.06

Where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{hour n}} - \text{Est.init.conc.}_{\text{hour 0}}) \times 100}{\text{Est.init.conc.}_{\text{hour 0}}}$$

Table 47 Long-term Stability of HPLC Analytical Method for Determination of Stavudine in Plasma

Time (Week)	Concentration (ng/ml)	Estimated Concentration (ng/ml)	Mean Estimated Concentration (ng/ml)	S.D.	%Deviation
0	100	93.691 93.529 93.448	93.556	0.124	-
	1750	1669.059 1668.765 1668.987	1668.937	0.153	-
2	100	91.387 90.953 91.098	91.146	0.221	-2.58
	1750	1645.537 1645.774 1644.644	1645.318	0.596	-1.42
4	100	90.25 91.147 91.582	90.993	0.679	-2.74
	1750	1640.555 1640.394 1638.671	1639.873	1.044	-1.74
6	100	91.091 89.591 90.339	90.340	0.750	-3.44
	1750	1627.178 1625.885 1625.347	1626.137	0.941	-2.56
8	100	90.603 90.477 89.295	90.125	0.722	-3.67
	1750	1609.251 1611.112 1611.91	1609.758	1.364	-3.55

Where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{week n}} - \text{Est.init.conc.}_{\text{week 0}}) \times 100}{\text{Est.init.conc.}_{\text{week 0}}}$$

2.3 Freeze-thaw stability

The freeze-thaw stability of stavudine in plasma data is presented in Table 48. Quality control samples were analysed immediately after preparation and after finishing three freeze-thaw cycles. The result showed that the percent deviation from the zero time of low concentration and high concentration of stavudine were –7.94% and -1.42%, respectively. These results indicated that the degradation of stavudine under three freeze-thaw cycles was within acceptance criteria (±15%), referring plasma samples could withstand to this stress condition.

2.4 Post-preparative stability

The stability of processed plasma samples ready for injection were analysed after freshly preparating, and after being kept in the autosampler for 4, 8, 12 and 16 hours.

Table 49 shows that the percent deviation from the zero time of low concentration and high concentration of stavudine were –5.74 to -24.75% and –3.29 to -20.83%, respectively. The results also show that all concentration of processed plasma samples are stable up to 8 hours. However, percent deviation from the zero time of low concentration and high concentration of stavudine were higher than 15% when processed plasma samples were kept in the autosampler for 12 and 16 hours, respectively. This result comply with the stability results of short-term room temperature which stated that stavudine tended to degrade rapidly at room temperature and so that each run of stavudine sample analysis should be finished within 8 hours.

Table 48 Freeze-thaw Stability of HPLC Analytical Method for Determination of Stavudine in Plasma

Cycle	Known Concentration (ng/ml)	Estimated Concentration (ng/ml)	Mean Estimated Concentration (ng/ml)	S.D.	%Deviation
0	100	93.513 91.751 92.857	92.707	0.891	-
	1750	1760.103 1758.745 1758.099	1758.982	1.023	-
3	100	84.695 85.319 86.002	85.339	0.654	-7.94
	1750	1735.387 1733.287 1733.499	1734.058	1.156	-1.42

Where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{cycle3}} - \text{Est.init.conc.}_{\text{cycle0}}) \times 100}{\text{Est.init.conc.}_{\text{cycle0}}}$$

Table 49 Post-preparative Stability of HPLC Analytical Method for Determination of Stavudine in Plasma

Time (hour)	Concentration (ng/ml)	Estimated Concentration (ng/ml)	Mean Estimated Concentration (ng/ml)	S.D.	%Deviation
0	100	92.112 90.987 91.645	91.581	0.565	-
	1750	1663.769 1662.056 1662.361	1662.729	0.914	-
4	100	86.781 86.155 86.049	86.328	0.396	-5.74
	1750	1607.848 1608.584 1607.439	1607.957	0.580	-3.29
8	100	81.315 82.261 80.728	81.435	0.773	-11.08
	1750	1536.244 1534.037 1534.166	1534.816	1.239	-7.69
12	100	77.828 76.081 77.307	77.072	0.897	-15.84
	1750	1419.397 1416.832 1419.055	1418.428	1.393	-14.69
16	100	69.336 69.485 67.923	68.915	0.862	-24.75
	1750	1315.465 1316.241 1317.590	1316.432	1.075	-20.83

Where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{hour n}} - \text{Est.init.conc.}_{\text{hour 0}}) \times 100}{\text{Est.init.conc.}_{\text{hour 0}}}$$

This assay validation study indicated that the analysis methods of stavudine in plasma samples had been proven to be reliable, specific, accurate and precise with the need of internal standard. The lower limit of quantification and stability data of this finding allowed to be successfully applied in a pharmacokinetic study of stavudine products.

3. Pharmacokinetics of stavudine in healthy rabbits

3.1 Plasma stavudine concentration-time profile

Twelve healthy rabbits participated in this study. None withdrew from the study or exhibited signs of adverse drug reactions to stavudine.

The plasma stavudine concentration at various times from 12 subjects following oral administration of 100 mg single dose of test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR) are summarized in Tables 50 to 51.

The result showed that there was rapid absorption of stavudine for Zerit® IR and the absorption of stavudine was slower for d4T pellet. It was shown that stavudine concentrations were reached to maximum within 0.5-0.75 hour and 3-3.5 hours from Zerit® IR and stavudine pellet, respectively. After reaching C_{max}, plasma concentrations of the drug from Zerit® IR and d4T pellet declined reach the undetectable level of stavudine at the end of 6 hours and 24 hours, respectively.

The chromatogram of plasma drug concentration of rabbits after oral administration of stavudine pellet is presented in Figures 44.

The individual plasma stavudine concentration time-profiles of two preparations from each subject are displayed graphically from Figures 45 to 46. A comparison of the mean plasma stavudine concentration time-profile between two preparations is present in Figure 47.

Table 50 Plasma Concentration (ng/ml) of stavudine from 12 Subjects Following Oral Administration of 100 mg single dose of stavudine pellets

Time (hr)	Subject No.												Mean	SD	%CV	Min	Max	Min-Max
	1	2	3	4	5	6	7	8	9	10	11	12						
0.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
1.00	51.37	*	52.21	72.83	69.03	*	58.22	*	*	54.21	64.57	*	60.35	8.54	14.16	51.37	72.83	21.46
2.00	127.12	103.33	160.64	242.45	180.06	136.94	140.92	141.25	131.37	129.42	140.71	131.37	147.13	35.29	23.99	103.33	242.45	139.12
2.50	240.03	177.28	258.13	444.53	318.46	242.43	274.87	320.65	342.46	301.17	336.82	342.46	299.94	68.07	22.70	177.28	444.53	267.25
3.00	362.94	376.31	376.95	656.27	456.45	448.89	546.09	568.31	572.93	575.31	516.67	572.52	502.47	96.01	19.11	362.94	656.27	293.33
3.25	582.00	469.45	454.47	638.97	560.11	648.46	620.59	632.27	674.84	585.66	505.44	664.41	586.39	75.16	12.82	454.47	674.84	220.37
3.50	602.41	508.23	504.61	576.62	571.54	636.52	603.40	619.94	659.74	564.46	495.12	659.65	583.52	57.81	9.91	495.12	659.74	164.62
3.75	564.32	475.25	493.87	520.16	493.22	567.97	532.13	558.96	648.56	515.45	458.15	648.15	539.68	61.41	11.38	458.15	648.56	190.41
4.00	487.00	455.13	453.71	466.63	411.07	528.50	453.02	477.00	580.43	465.43	417.04	580.04	481.25	55.29	11.49	411.07	580.43	169.36
6.00	301.02	290.71	254.16	285.65	243.73	329.13	250.34	284.38	326.56	309.56	234.21	326.21	286.31	33.95	11.86	234.21	329.13	94.92
9.00	191.09	160.88	138.32	162.83	120.00	176.54	140.78	160.00	176.21	173.21	141.33	202.17	161.95	23.75	14.67	120.00	202.17	82.17
12.00	125.89	89.82	91.72	109.65	80.00	103.72	94.05	96.00	89.13	113.13	79.71	109.62	98.54	14.05	14.25	79.71	125.89	46.18
15.00	75.86	50.12	55.63	74.13	58.88	62.46	64.78	67.61	67.09	75.09	54.26	81.09	65.58	9.71	14.81	50.12	81.09	30.97
18.00	*	*	*	51.29	*	*	*	*	*	56.23	*	54.58	54.03	2.51	4.65	51.29	56.23	4.94
21.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
24.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
30.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-

*Drug concentration < LLOQ (LLOQ = 50 ng/ml)

Table 51 Plasma Concentration (ng/ml) of stavudine from 12 Subjects Following Oral Administration of 50 mg single dose of Zerit® IR

Time (hr)	Subject No.												Mean	SD	%CV	Min	Max	Max-Min
	1	2	3	4	5	6	7	8	9	10	11	12						
0.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
0.25	308.65	457.78	605.04	294.99	498.82	392.73	421.65	369.88	518.82	326.63	608.36	441.24	437.05	106.21	24.30	442.49	912.54	470.05
0.50	930.59	1259.97	1228.06	1114.21	1124.55	984.01	992.83	894.93	1037.39	1111.24	1195.78	1058.95	1077.71	115.77	10.74	1342.40	1842.09	499.69
0.75	1089.85	906.72	1059.84	1262.89	984.45	1174.93	1025.22	1129.87	1244.91	971.77	1116.27	1169.09	1094.65	110.04	10.05	1360.08	1894.34	534.26
1.00	835.61	661.62	724.29	963.65	629.03	931.64	754.53	832.21	1057.69	749.85	813.37	950.53	825.33	130.26	15.78	943.54	1586.54	643.00
1.50	435.47	257.13	379.01	553.89	314.93	520.81	385.73	415.65	578.63	419.31	345.73	554.51	430.07	102.84	23.91	385.69	867.95	482.26
2.00	280.25	116.57	200.86	324.61	176.50	267.38	211.60	231.15	300.91	265.23	162.02	371.26	242.36	73.11	30.17	174.86	556.89	382.03
3.00	145.43	46.95	97.28	157.09	89.15	137.30	105.61	95.32	134.01	150.19	62.86	206.35	118.96	44.47	37.39	46.95	206.35	159.40
4.00	53.87	*	*	*	*	55.08	*	*	*	63.85	*	75.94	62.19	10.19	16.39	53.87	75.94	22.07
6.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
9.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-
12.00	*	*	*	*	*	*	*	*	*	*	*	*	-	-	-	-	-	-

*Drug concentration < LLOQ (LLOQ = 50 ng/ml)

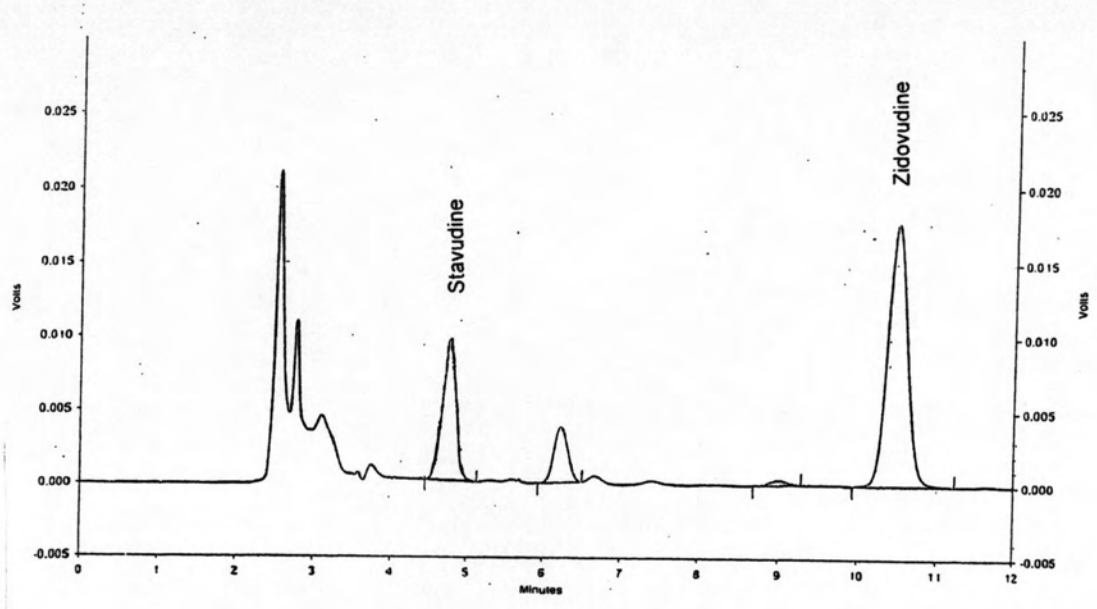


Figure 44 Chromatogram of Plasma Drug Concentration obtained from Subject No.9 after oral administration of stavudine pellets for 3.50 hours

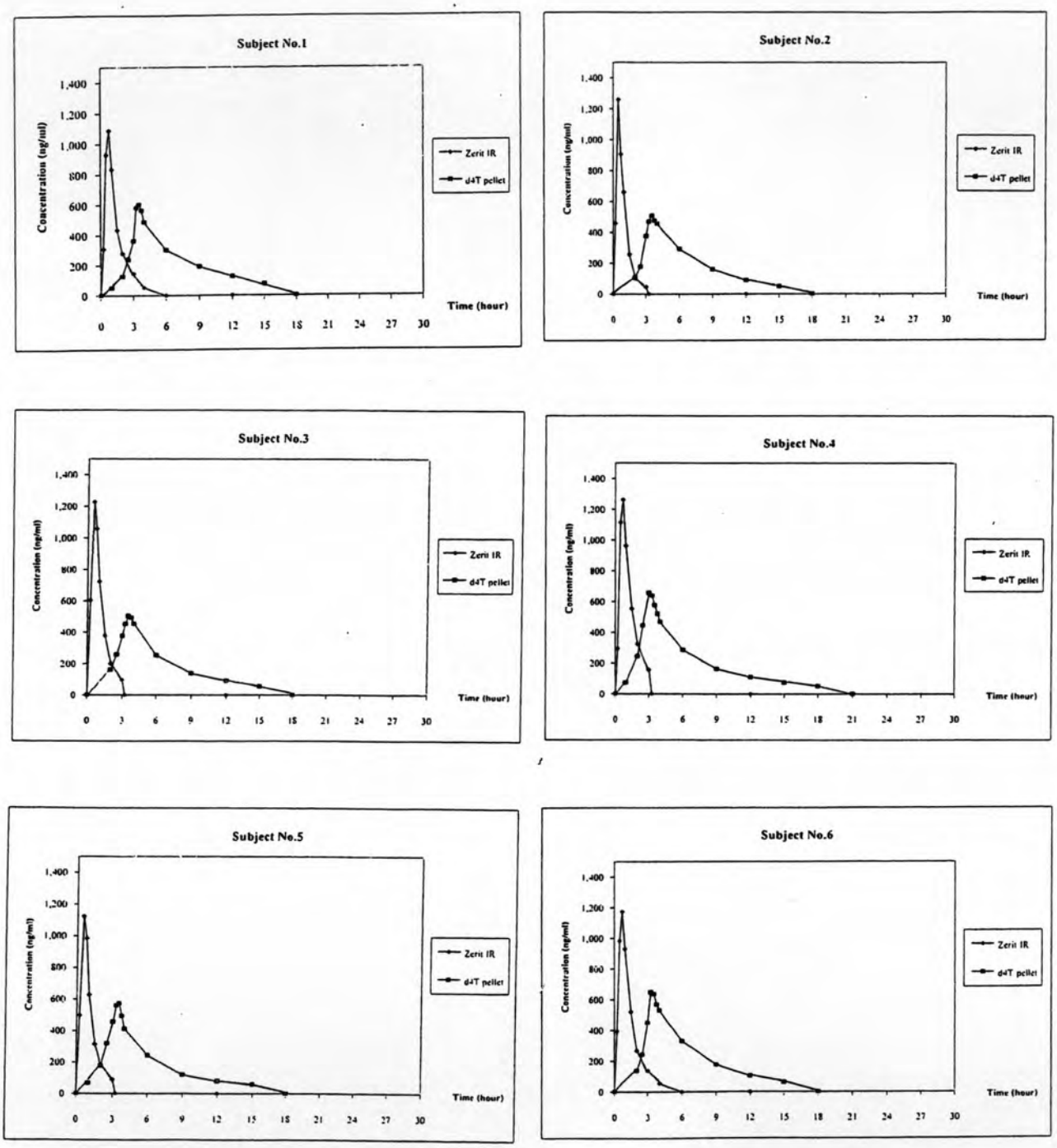


Figure 45 Plasma Stavudine Concentration-time Profiles of Individual Subject No.1-6 Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

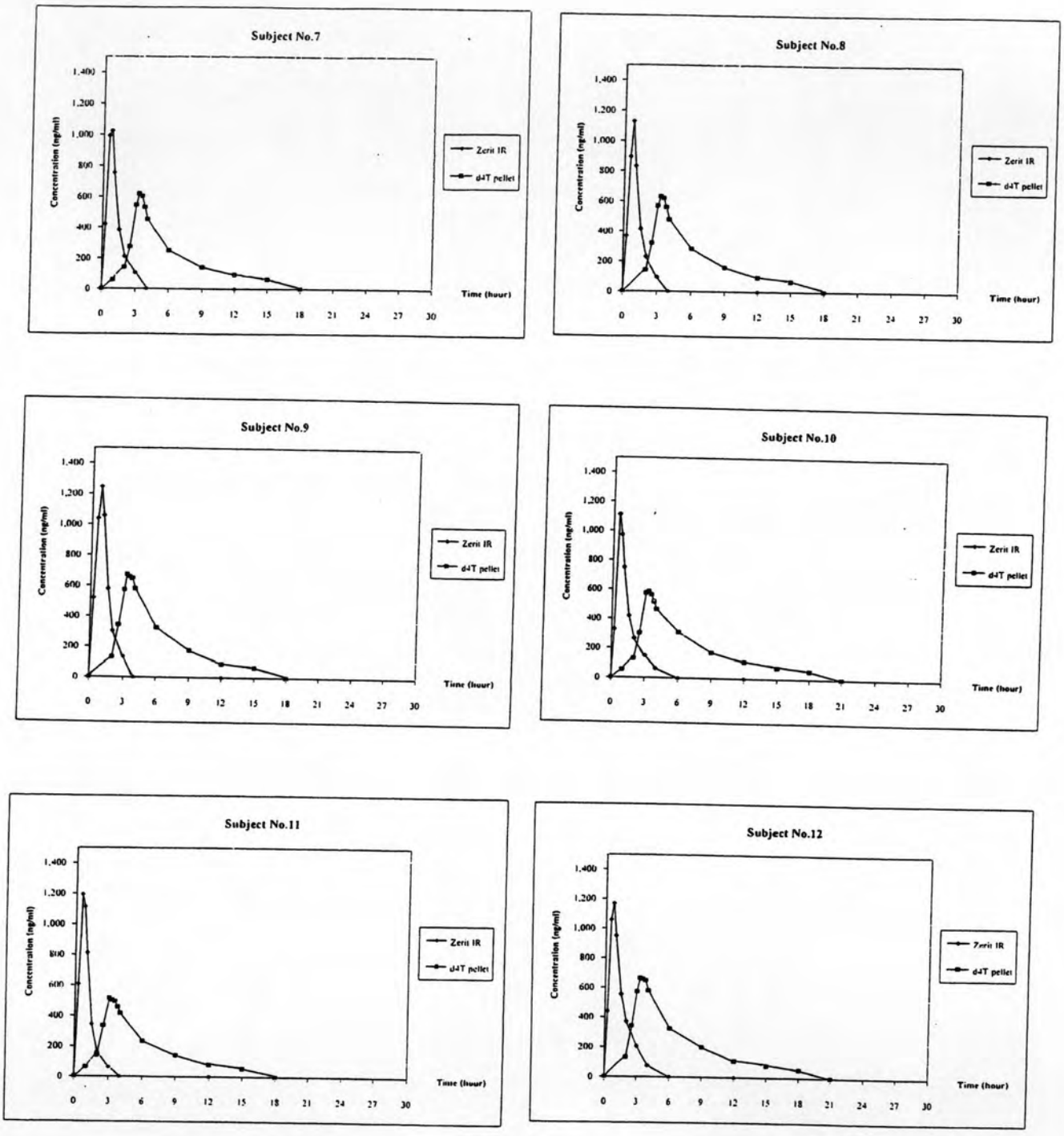


Figure 46 Plasma Stavudine Concentration-time Profiles of Individual Subject No.7-12 Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

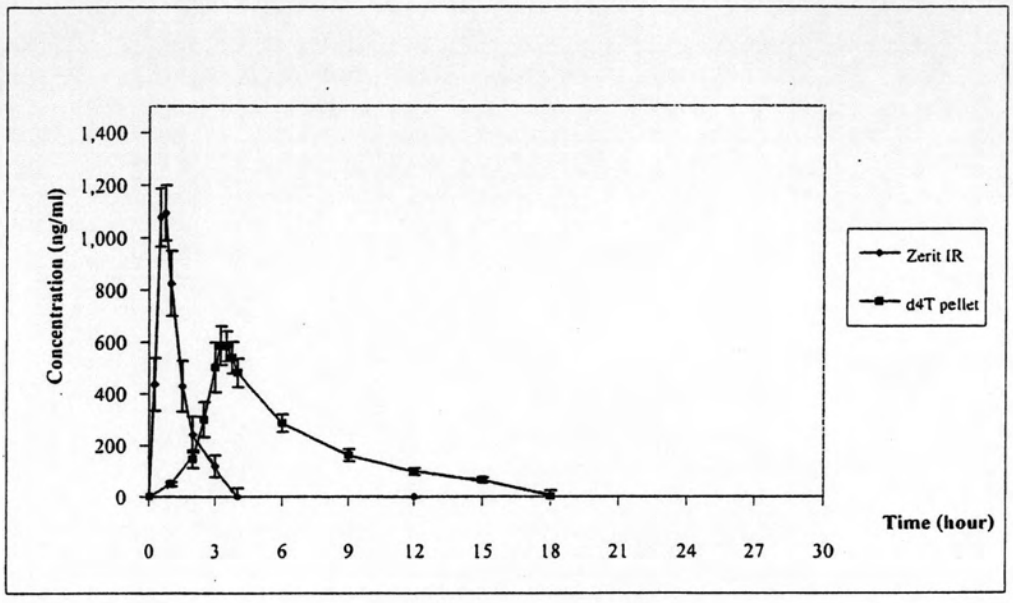


Figure 47 Mean Plasma Stavudine Concentration-time Profiles of 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

3.2 Pharmacokinetics analysis

Since stavudine was absorbed very rapidly and although blood samples were collected every 15 minutes, some peak plasma concentrations of innovator's product have been occurred less than 30 minutes. The amount of collected data points were not enough for calculating drug absorption rate constant (K_a) in this study. The primary pharmacokinetic parameters ; AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were analysed and shown in Tables 52 to 53.

Other related pharmacokinetic parameters; t_{max}, elimination rate constant (k_e), elimination half life ($t_{1/2}$) and percentage relative bioavailability of stavudine from all subjects participated in this study were also obtained and presented in Tables 54 to 57. Regarding the difference of these parameters were performed.

Elaboration of these principally relevant pharmacokinetic parameters obtained for bioavailability comparison were as follows :

3.2.1 Area under the plasma stavudine concentration-time curves (AUC_{0-t} and $AUC_{0-\infty}$)

The AUC_{0-t} and $AUC_{0-\infty}$ of stavudine for test and innovator's product from all subjects are present in Table 52. To ensure a reliable estimation of the extent of absorption, a collection period of at least three half-life is recommended by Thai Food and Drug Administration. All subjects had an AUC_{0-t} /$AUC_{0-\infty}$ ratio > 80% for stavudine and the mean extrapolated value was not more than 20%, indicating the sampling scheme was sufficiently long to ensure an adequate description of the absorption phase and also fully characterized the pharmacokinetic properties of stavudine.

After oral administration of stavudine pellet 100 mg and Zerit® IR 50 mg, the AUC_{last} values for test and innovator's product were 3,214.09 and 1,504.79 ng.hr/ml, respectively. The $AUC_{0-\infty}$ values for test and innovator's product were 3,585.96 and

1,606.21 ng.hr/ml, respectively. These results indicated that extent of drug absorption for test product was two fold higher than that of innovator's product complying with dose of stavudine pellet given which was double of dose of Zerit® IR.

3.2.2. Peak plasma stavudine concentration (C_{max})

The C_{max} values of stavudine for test and innovator's products were 598.83 and 1168.03 ng/ml, respectively as shown in Table 53. These results indicated that the rate of drug adsorption for test and innovator's products were different. It can be explained by the fact that the difference of pharmaceutical dosage form is a major effect on the rate of drug absorption. The test product is the control release pellet which can retard and prolong drug release, it is possibility that the rate of drug absorption was slower than Zerit® IR capsule which can be dissolved and absorbed rapidly. In addition, the difference of drug absorption rate possibly depends on the pharmaceutical formulation, physicochemical properties of drug (particle size, crystal form) or inactive ingredient in formulation. In this study, inactive ingredient in stavudine pellet was only microcrystalline cellulose whereas there is the report by BMS company to support that each capsule of Zerit® IR contains microcrystalline cellulose, sodium starch glycolate, lactose and magnesium stearate. These ingredients might affect absorption of drug.

3.2.3 Time to peak plasma stavudine concentration (t_{max})

The time to peak plasma concentrations of stavudine for test and innovator's product were 3.29 and 0.63 hours, respectively as presented in Table 54. This indicated that innovator's product was rapidly absorbed and the t_{max} values of stavudine of test product were very higher than those of innovator's product. The difference of t_{max} of test product to that of innovator's products was 422.22%. These results indicated that the rate of drug adsorption for test and innovator's products were quite different..

The factors which affected this parameter were the same as those of C_{max} values.

3.2.4 Related pharmacokinetic parameters

The elimination rate constant (k_e) values for test and innovator's product were 0.1612 and 0.9375 hr^{-1}, respectively as present in Table 55.

The half life ($t_{1/2}$) values for test and innovator's product were 4.38 hr and 0.77 hr, respectively as present in Table 56.

These results demonstrated that the drug from stavudine pellet was eliminated slower and could be maintained in plasma longer than that of Zerit® IR. Because the stavudine immediate release dosage form is rapidly eliminated and the drug may not be accumulated in the body if the excretion process is in good condition, therefore this study design, two weeks washout period was sufficient to prevent carry over effect which may disturb the result of bioavailability study.

The percentage relative bioavailability value of stavudine in rabbit was 112.44% as present in Table 57. This result indicated that although stavudine pellet had lower C_{max} and higher T_{max} than those of Zerit® IR, the drug was completely absorbed into systemic circulation of rabbits and no problem of drug absorption.

3.3 Comparison of pharmacokinetics parameters of stavudine

The comparison of pharmacokinetics parameters between stavudine pellet and Zerit® IR is presented in Table 58. It can be concluded that the extent of drug absorption for stavudine pellet was well when compared with that of Zerit® IR as shown that 100 mg of stavudine were completely absorbed and AUC also was two fold higher than those of 50 mg Zerit® IR. However, the rate of absorption for stavudine pellet was less than that of Zerit® IR as shown that stavudine pellet had lower C_{max} and higher T_{max} although dose of stavudine pellet were double of Zerit® IR. Stavudine pellet also had higher $t_{1/2}$ than that of Zerit® IR which indicated that stavudine pellet can be maintained in plasma longer than Zerit® IR. All results supported the assumption that stavudine pellet dosage form can be an extended release product and this pharmaceutical dosage form had good trend to reduce the risk of dose dumping as well.

Table 52 Area Under the Plasma Stavudine Concentration-time Curves (AUC_{0-t} and $AUC_{0-\infty}$) of 12 Subjects Following Oral Administration 100 mg single dose of Test Product (Stavudine pellets) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	Innovator's Product		Test Product	
	AUC_{last} (ng.hr/ml)	AUC_{∞} (ng.hr/ml)	AUC_{last} (ng.hr/ml)	AUC_{∞} (ng.hr/ml)
1	1549.78	1615.79	3318.96	3819.03
2	1120.22	1189.64	2863.41	3120.17
3	1432.27	1542.73	2809.18	3145.11
4	1706.88	1897.57	3589.76	3988.59
5	1266.83	1375.72	2789.11	3167.52
6	1668.14	1732.16	3369.51	3709.15
7	1350.05	1475.85	2979.49	3348.13
8	1387.26	1485.73	3221.20	3573.89
9	1745.92	1886.78	3512.75	3883.41
10	1538.05	1625.68	3484.48	3930.04
11	1392.36	1449.02	2777.54	3105.79
12	1899.73	1997.86	3853.64	4240.73
Mean	1504.79	1606.21	3214.09	3585.96
S.D.	222.58	237.46	364.42	397.82
%C.V.	14.79	14.78	11.34	11.09
Min	1120.22	1189.64	2777.54	3105.79
Max	1899.73	1997.86	3853.64	4240.73
Max-Min	779.51	808.22	1076.10	1134.94

Table 53 Peak Plasma Concentrations of stavudine (C_{max}) from 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	C_{max} (ng/ml)	
	Innovator's Product	Test Product
1	1089.85	602.41
2	1259.97	508.23
3	1228.06	504.61
4	1262.89	656.27
5	1124.55	571.54
6	1174.93	648.46
7	1025.22	620.59
8	1129.87	632.27
9	1244.91	674.84
10	1111.24	585.66
11	1195.78	516.67
12	1169.09	664.41
Mean	1168.03	598.83
S.D.	74.38	61.94
%C.V.	6.37	10.34
Min	1537.83	504.61
Max	1894.34	674.84
Max-Min	356.51	170.23

Table 54 Time to Peak Plasma Concentrations of Stavudine (t_{max}) from 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	t_{max} (hr)	
	Innovator's Product	Test Product
1	0.75	3.50
2	0.50	3.50
3	0.50	3.50
4	0.75	3.00
5	0.50	3.50
6	0.75	3.25
7	0.75	3.25
8	0.75	3.25
9	0.75	3.25
10	0.50	3.25
11	0.50	3.00
12	0.75	3.25
Mean	0.63	3.29
S.D.	0.12	0.17
%C.V.	19.58	5.22
Min	0.50	3.00
Max	0.75	3.50
Max-Min	0.25	0.50

Difference of t_{max} values of test vs. innovator's product $= \frac{(3.29-0.63)}{0.63} \times 100$

$= 422.22\ \%$

Table 55 Elimination rate constant; k_e of Stavudine from 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	k_e (hr^{-1})	
	Innovator's Product	Test Product
1	0.8161	0.1517
2	1.6793	0.1952
3	0.8807	0.1656
4	0.8238	0.1286
5	0.8187	0.1556
6	0.8603	0.1839
7	0.8395	0.1486
8	0.9680	0.1917
9	0.9514	0.1810
10	0.7286	0.1262
11	1.1094	0.1653
12	0.7739	0.1410
Mean	0.9375	0.1612
S.D.	0.25	0.02
%C.V.	27.14	14.50
Min	0.7286	0.1262
Max	1.6793	0.1952
Max-Min	0.9507	0.0690

Table 56 Elimination half life; $t_{1/2}$ of Stavudine from 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	$t_{1/2}$ (hr)	
	Innovator's Product	Test Product
1	0.85	4.57
2	0.41	3.55
3	0.79	4.18
4	0.84	5.39
5	0.85	4.45
6	0.81	3.77
7	0.83	4.66
8	0.72	3.62
9	0.73	3.83
10	0.95	5.49
11	0.62	4.19
12	0.90	4.91
Mean	0.77	4.38
S.D.	0.14	0.65
%C.V.	18.43	14.88
Min	0.41	3.55
Max	0.95	5.49
Max-Min	0.54	1.94

Table 57 Percentage Relative bioavailability of Stavudine from 12 Subjects Following Oral Administration of 100 mg single dose of Test Product (stavudine pellet) and 50 mg single dose of Innovator's Product (Zerit® IR)

Subject no.	Relative bioavailability (%)
1	118.18
2	131.14
3	101.93
4	105.10
5	115.12
6	107.07
7	113.43
8	120.27
9	102.91
10	120.87
11	107.17
12	106.13
Mean	112.44
S.D.	8.92
%C.V.	7.93
Min	101.93
Max	131.14
Max-Min	29.21

Table 58 Summary of Pharmacokinetic Parameters of Stavudine ($\bar{X} \pm$ S.D.) in rabbits

Pharmacokinetic parameters	Stavudine pellet	Zerit® IR
AUC_{last} (ng.hr/ml)	3214.09 ± 364.42	1504.79 ± 222.58
AUC_{∞} (ng.hr/ml)	3585.96 ± 397.82	1606.21 ± 237.46
C_{max} (ng/ml)	598.83 ± 61.94	1168.03 ± 74.38
t_{max} (hr)	3.29 ± 0.17	0.63 ± 0.12
Relative bioavailability (%)	112.44 ± 8.92	-

Consideration about the comparison of the results in this study with the results in other reports, because stavudine controlled release dosage form (Zerit® XR 100 mg) is a new drug and it is still under the clinical trial studies, therefore Zerit® XR 100 mg cannot be found in the market. All reports studied pharmacokinetic parameters of stavudine immediate release (Zerit® IR) in human plasma and the studies for stavudine controlled release dosage form were only reported by Bristol Mayer Squibb Company (BMS). However, no report studied in rabbits and so that it is difficult to exactly compare pharmacokinetic parameters from this study with the previous reports by the BMS company due to there are some various factors affected by the different kind of animals.

To compare pharmacokinetic parameters of the extend release dosage form with those reports and also study whether the plasma drug concentration level from stavudine pellet can be reached the therapeutic level, studying pharmacokinetic parameters of stavudine pellet in human volunteers in the future was required.

3.4 Photomicrograph of stavudine pellet collected from feces of rabbit

In this study, it was observed that stavudine from Zerit® IR was eliminated rapidly and the plasma stavudine concentration of stavudine pellet was also declined within 8 hours, therefore feces of rabbit were collected at several times for investigation. The stavudine pellets were first found in feces after 8 hours of oral administration of test product.

Photomicrographs of stavudine pellet collected from feces of rabbit are shown in Figure 48. It showed that film coating layer of stavudine pellet was destroyed and formed so many pores on the surface of pellets. This result indicated that the control release property of stavudine pellets might be lost by the enzymes or the movement of GI tract of rabbits. In addition, it is possibility that the GI tract of rabbits is shorter than that of a human including the half life of stavudine is also very short. Therefore, that might be the reason why stavudine pellets were eliminated from GI tract of rabbits in a short time and it cannot retard stavudine covering 24 hours. Since the film coating layer had been destroyed, it was probability that whole drug in coated pellets were released. However, few pellets were found in feces of rabbit and amount of pellets found was not enough to assay drug content. Therefore, this assumption was proved by the previous results respect to the extent of drug absorption which illustrated that 100 mg of stavudine pellet were completely absorbed and AUC also was two fold higher than those of 50 mg Zerit® IR. The elimination rate constant and mean half life of stavudine pellets also indicated that stavudine pellet can extend drug release longer than Zerit® IR although the film was destroyed. Therefore, this product has good trend to be developed and studied in human in the future.

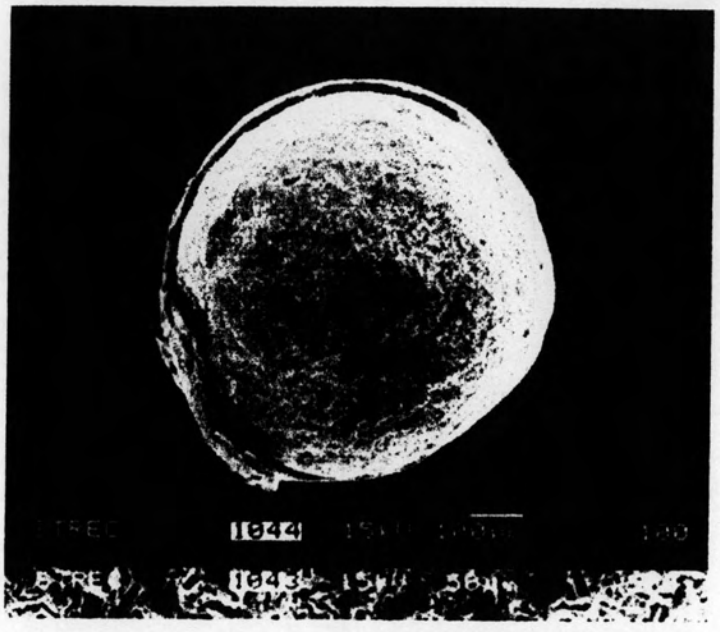


Figure 48 Stavudine pellet collected from feces of rabbit after 8 hours of oral administration of test product

(A. Stavudine pellet X 100 magnifying

B. Stavudine pellet X 500 magnifying)