CHAPTER III

RESULTS AND DISCUSSION

1. Formulation of ketoprofen rectal suppositories.

Three conventional hydrophilic suppository base used in this study were called " Base 1, Base 2 and Base 3 ", respectively. The compositions of each base were two polyethylene glycol with different molecular weight at specified amount. One commercially available conventional hydrophobic suppository base was also included for comparison and called Suppocire® AM. The matrix rectal suppositories were formulated using various proportion of ketoprofen to prolonged release carrier weight by weight.

1.1 Determination of the displacement value (D.V.)

The displacement value is the weight in gram of substance which can substitute 1 gram of suppository base, for example, the displacement value of ketoprofen in Base 1 was 1.427 meant that 1.427 gram of ketoprofen could substitute 1 gram of Base 1. All displacement values as shown in Table 4 were obtained by determination of 10 samples.

Table 4 The displacement values of each compositions in suppository bases.

The displacement value of	Base 1	Base 2	Base 3	Suppocire® AM
Ketoprofen	1.427	1.188	1.008	1.109
Eudragit S-100	1.312	1.147	2.564	-
HP55	1.822	1.266	3.300	-

Data of Table 4 revealed that the displacement values of ketoprofen, Eudragit S-100 and HP55 were different in each individual suppository base. Reasons might be due to the property of each ingredient, the compositions of all ingredients as well as its weight or amount in the formula of each base were all different. Besides, difficulty of preparing to achieve the exact suppository might contribute to these differences.

Whenever the displacement values had been determined, the amount of suppository base to be used in each formulation could correctly been calculated.

1.2 Preparation of ketoprofen rectal suppositories.

During the preparation of suppositories, some difficulties were experienced in achieving the exact dosage. There were no problems in preparing suppositories from Suppocire® AM and Eudragit S-100. This was because the compositions were easily melted. The viscosity was not increased and the suppository was easily dislodged from the mold.

Higher temperature and longer time were needed in order to melt hydroxypropyl methylcellulose phthalate (HP55). Since it was derived from hydroxypropyl methylcellulose (HPMC). Thus, during melting, its viscosity was increased and became sticky. Preparing was difficult to achieve the uniformity of content and lubrication was necessary to remove suppositories from the molds. The greater amount of HP55 was used, the more difficult for preparing was seen.

1.3 Evaluation of physical and chemical properties of suppositories.

1.3.1 Physical appearance of rectal suppositories.

All suppositories prepared from hydrophilic bases were white whereas those prepared from hydrophobic one were pale yellow. Suppository with

Eudragit S-100 gradually dissolved in room temperature. So did the one with hydroxypropyl methylcellulose phthalate and it also became sticky. Suppositories prepared from Suppocire® AM gradually melted in room temperature because its drop point ranged between 35-36.5°C.

1.3.2 Uniformity of weight and uniformity of content of conventional rectal suppositories.

The average weight of each suppository for Base 1, Base 2 and Base 3 were 2.4450 ± 0.0122, 2.4508 ± 0.0122 and 2.5095 ± 0.0196 g, respectively, whereas that of Suppocire ®AM was 1.9707 ± 0.0159 g as presented in Table 5. All formulations conformed the specification for uniformity of weight of the BP 1993.

The differences of average weight of these suppositories were resulted from the differences of molecular weight among Base 1, Base 2, Base 3 and Suppocire ®AM. Because each individual mold was capable of holding a specific volume of material in each of its openings, so the higher molecular weight of the base was used, the greater average weight of suppository was achieved. As seen by the compositions of Base 3 that were the highest molecular weight, so its average weight of suppository was the greatest. Suppocire ®AM were mixtures of triglyceride esters of the higher saturated fatty acids along with varying proportions of mono- and diglycerides. Its molecular weight was less than those of Base 1, Base 2 and Base 3. Thus the average weight of suppositories prepared from Suppocire ®AM was also less than those obtained using the bases mentioned.

The uniformity of content of ketoprofen in each suppository stated as percent labeled amount (%L.A.) was shown in Table 6. They were 93.54 ± 1.07, 94.59 ± 1.72, 92.33 ± 1.70 and 97.20 ± 3.63 percent for Base 1, Base 2, Base 3 and Suppocire ®AM, respectively. All formulations met the acceptable limits of BP 1993. Although the USP did not specify the content uniformity standards for

suppositories, these were determined and found tc be within 100 ± 10% of the labeled amount of ketoprofen in all suppositories.

1.3.3 Uniformity of weight and uniformity of content of matrix rectal suppositories.

In this study, the prolonged release ketoprofen rectal suppositories were divided into 2 classes depending on types of prolonged release (poorly water soluble) carrier incorporated in conventional hydrophilic suppository bases

Class I Ketoprofen : Eudragit S -100 ratios (weight by weight) = 1:1, 1:1.5 and 1:2.

Class II Ketoprofen : HP55 ratios (weight by weight) = 1:3 and 1:4.

The reasons of selecting these five ratios were according to the facts that:

1. The objective of formulation was to provide prolonged release of ketoprofen with minimum utilization of prolonged release carrier. This was achieved by observing the time consumed to completely release of the drug from dissolution studies.

2. The possibility of preparing that formulation was concerned. From preliminary studies, these ratios were found to be the most appropriate and could be prepared using the available equipments in the laboratory of Pharmacy Department.

3. Ketoprofen : HP55 ratio was reported previously (Ermis and Tarimci, 1995).

Table 5 Weight of each suppository (g) from three formulations of conventional hydrophilic and one conventional hydrophobic ketoprofen rectal suppositories.

Suppositories	Hydrophilic			Hydrophobic
No.	Base 1	Base 2	Base 3	Suppocire®AM
1	2.4293	2.4409	2.5058	1.9926
2	2.4462	2.4401	2.4760	1.9753
3	2.4441	2.4465	2.5063	1.9835
4	2.4429	2.4224	2.4911	1.9807
5	2.4551	2.4405	2.5027	1.9982
6	2.4646	2.4695	2.5497	1.9488
7	2.4583	2.4707	2.5416	1.9926
8	2.4505	2.4526	2.5027	1.9979
9	2.4423	2.4403	2.5080	1.9612
10	2.4666	2.4394	2.4881	1.9757
11	2.4695	2.4482	2.5279	1.9523
12	2.4501	2.4449	2.5096	1.9520
13	2.4373	2.4540	2.5219	1.9549
14	2.4324	2.4596	2.4949	1.9707
15	2.4441	2.4484	2.5031	1.9705
16	2.4430	2.4562	2.5105	1.9677
17	2.4374	2.4642	2.5121	1.9637
18	2.4337	2.4541	2.5477	1.9566
19	2.4321	2.4550	2.4955	1.9651
20	2.4353	2.4653	2.4949	1.9546
Mean	2.4450	2.4508	2.5095	1.9707
S.D.	0.0122	0.0122	0.0196	0.0159

Table 6 Uniformity of content of ketoprofen (%L.A.) from four formulations of conventional ketoprofen rectal suppositories.

Suppositories No.	% L.A. of Ketoprofen			
	Base 1	Base 2	Base 3	Suppocire® AM
1	93.23	98.17	93.86	100.70
2	92.59	94.94	93.70	97.63
3	94.12	92.79	95.26	99.97
4	95 10	93.10	91.99	98.50
5	93.35	94.48	93.55	102.61
6	92.13	94.64	90.59	94.25
7	93.26	92.33	90.12	98.36
8	92.89	94.64	92.15	94.69
9	95.22	94.48	91.52	94.84
10	92.78	96.33	90.59	90.44
Mean	93.54	94.59	92.33	97.20
S.D.	1.07	1.72	1.70	3.63

1.3.3.1 Ketoprofen : Eudragit S-100 ratios

Average weights and uniformity of contents of ketoprofen in each base of suppositories prepared using three different ratios between the drug and Eudragit S-100 were presented in Table 7-8. Both values conformed the specifications of BP1993. The average weight of suppository from all ratios were nearly the same values. However the weight of suppository of each ratio prepared from Base 3 appeared to be slightly greater than those of Base 1 and Base 2. These results agreed well with the weight of suppository base as shown in Table 5.

1.3.3.2 Ketoprofen : HP55 ratios.

Average weights and uniformity of contents of ketoprofen in each base of suppositories prepared using two different ratios of ketoprofen to hydroxypropyl methylcellulose phthalate were shown in Table 9-10. Results demonstrated that both values conformed the requirements of BP 1993. The average weight of suppository from all ratios were, again, almost the same values as found with the formulas using Eudragit S-100 as the prolonged release carrier. Suppositories prepared from Base 1 of both ratios showed greater average weight than others even if the displacement value of Base 1 was less than that of Base 3. This unexpected results might probably be due to Base 1 was composed of the larger amount of low molecular PEG than others. This made that base more hygroscopic resulted in higher weight gain.

1.4 Validation of analytical methods for *in vitro* studies.

Due to the analytical method for ketoprofen in suppositories was not available elsewhere. The spectrophotometry was, therefore, developed to be used in this study. It was validated for accuracy and precisions. In most cases, the precent recovery was to be almost 100 percent coefficient of variations for both within run and between run precisions of each concentration were less than 6 percent. All standard

Table 7 Weight of each suppository (g) from three formulations of prolonged release ketoprofen rectal suppositories using Eudragit S-100 as prolonged release carrier.

Suppositories	Ketoprofen : Eudragit S-100 = 1:1			Ketoprofen : Eudragit S-100 = 1:1.5			Ketoprofen : Eudragit S-100 = 1:2		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	2.4272	2.4509	2.5390	2.4414	2.4005	2.4617	2.4827	2.4512	2.4773
2	2.4491	2.4339	2.5445	2.4103	2.3625	2.4674	2.4879	2.4371	2.4972
3	2.4885	2.4430	2.5426	2.3786	2.3745	2.4877	2.4743	2.4599	2.5027
4	2.4396	2.4430	2.5261	2.3955	2.3867	2.4874	2.4541	2.4526	2.5064
5	2.4593	2.4452	2.5588	2.4112	2.3768	2.4857	2.4908	2.4452	2.4793
6	2.4304	2.4341	2.4907	2.4617	2.3962	2.4933	2.4785	2.4570	2.4807
7	2.4630	2.4214	2.5022	2.4743	2.4313	2.4773	2.4716	2.4520	2.4137
8	2.4538	2.4761	2.4980	2.4767	2.4186	2.4616	2.4605	2.4483	2.3939
9	2.4444	2.4467	2.4898	2.4687	2.4337	2.4828	2.4706	2.4632	2.4349
10	2.4537	2.4370	2.4991	2.4596	2.4478	2.5111	2.4780	2.4296	2.4375
11	2.4493	2.4455	2.5012	2.4530	2.3958	2.4923	2.4867	2.4378	2.4389
12	2.4236	2.4161	2.4937	2.4272	2.4201	2.5100	2.4695	2.4327	2.4209
13	2.4492	2.4747	2.5283	2.4512	2.4654	2.4321	2.4879	2.4550	2.4554
14	2.4524	2.4590	2.5115	2.4127	2.4387	2.4259	2.4714	2.4717	2.4196
15	2.4517	2.4613	2.5196	2.4517	2.4331	2.4382	2.4515	2.4552	2.4268
16	2.4753	2.4343	2.4923	2.4341	2.4463	2.3911	2.4705	2.4767	2.4325
17	2.4716	2.4462	2.5069	2.4446	2.4606	2.4089	2.4750	2.4571	2.4821
18	2.4587	2.4627	2.4625	2.4214	2.4323	2.4546	2.4829	2.4614	2.4630
19	2.4435	2.4549	2.4637	2.4520	2.4208	2.4720	2.4656	2.4328	2.4458
20	2.4256	2.4133	2.4595	2.4111	2.4430	2.4223	2.4863	2.4565	2.4964
Mean	2.4505	2.4450	2.5065	2.4354	2.4187	2.4632	2.4748	2.4517	2.4553
S.D.	0.0168	0.0171	0.0278	0.0279	0.0296	0.0336	0.0112	0.0128	0.0334

Table 8 Uniformity of content of ketoprofen (%L.A.) from three formulations of prolonged release ketoprofen rectal suppositories using Eudragit S-100 as prolonged release carrier.

Suppositories	Ketoprofen : Eudragit S-100 = 1:1			Ketoprofen : Eudragit S-100 = 1:1.5			Ketoprofen : Eudragit S-100 = 1:2		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	104.91	97.43	97.51	90.13	99.58	94.88	95.27	109.22	93.99
2	103.24	100.24	101.19	91.29	99.88	93.12	91.71	95.96	94.20
3	101.27	98.99	96.95	90.78	92.68	96.57	96.11	96.26	91.03
4	95.36	99.62	105.71	93.65	99.43	90.02	99.43	93.87	92.23
5	98.24	109.33	97.77	92.89	95.53	92.12	96.20	90.69	95.87
6	96.42	108.21	99.28	90.57	98.53	91.81	98.33	96.56	93.31
7	99.30	107.39	102.15	91.11	104.83	92.55	90.03	105.77	91.96
8	96.58	105.55	93.80	93.65	102.28	91.00	95.10	98.03	92.65
9	96.73	101.34	92.85	93.66	106.65	93.22	104.95	107.71	92.90
10	100.06	102.90	97.91	90.87	102.90	91.20	96.03	95.75	90.86
Mean	99.21	103.10	98.51	91.86	100.23	92.65	96.32	98.98	92.90
S.D.	3.17	4.24	3.82	1.43	4.17	1.94	4.11	6.29	1.53

Table 9 Weight of each suppository (g) from two formulations of prolonged release ketoprofen rectal suppositories using HP55 as prolonged release carrier.

Suppositories	Ketoprofen : HP55 = 1:3			Ketoprofen : HP55 = 1:4		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	2.5266	2.5366	2.5279	2.5127	2.4687	2.4758
2	2.5187	2.5292	2.5431	2.5114	2.4684	2.4932
3	2.5061	2.5214	2.5384	2.4946	2.4815	2.4125
4	2.5024	2.5340	2.4777	2.5275	2.4630	2.4899
5	2.5188	2.5351	2.4715	2.5265	2.4525	2.4716
6	2.5107	2.5449	2.4890	2.5106	2.4573	2.4749
7	2.4787	2.4659	2.4790	2.4968	2.3994	2.4605
8	2.4656	2.4489	2.4906	2.4900	2.4409	2.4600
9	2.5208	2.4466	2.4769	2.5022	2.4362	2.4634
10	2.4623	2.4598	2.4845	2.5227	2.4480	2.4503
11	2.4787	2.4721	2.4844	2.5196	2.4135	2.4372
12	2.4656	2.4697	2.4984	2.5196	2.4484	2.4604
13	2.4647	2.4963	2.5368	2.5216	2.4808	2.4503
14	2.5261	2.5006	2.5219	2.4996	2.4789	2.4480
15	2.5162	2.5038	2.4578	2.5232	2.4935	2.4503
16	2.5235	2.5024	2.4756	2.4845	2.4897	2.4327
17	2.5284	2.4934	2.4749	2.5077	2.4806	2.4793
18	2.5165	2.5111	2.4814	2.5006	2.4903	2.4533
19	2.4987	2.5044	2.4927	2.4978	2.4324	2.4564
20	2.4612	2.4754	2.5278	2.5123	2.4649	2.4821
Mean	2.4997	2.4976	2.4965	2.5091	2.4594	2.4600
S.D.	0.0249	0.0305	0.0265	0.0128	0.0258	0.0197

Table 10 Uniformity of content of ketoprofen (%L.A.) from two formulations of prolonged release ketoprofen rectal suppositories using HP55 as prolonged release carrier.

Suppositories	Ketoprofen : HP55 = 1:3			Ketoprofen : HP55 = 1:4		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	93.44	90.29	93.72	106.67	102.82	106.99
2	91.79	91.04	96.60	106.03	107.63	103.78
3	94.49	90.14	94.20	106.99	108.91	93.21
4	94.94	91.34	94.68	106.99	108.91	109.87
5	91.34	94.19	94.52	105.64	106.99	100.58
6	90.89	96.44	100.29	108.27	105.06	105.06
7	93.14	92.84	97.72	101.22	108.27	107.95
8	90.59	92.54	95.16	109.19	104.42	97.37
9	93.14	93.44	93.40	105.06	109.19	105.06
10	90.59	91.64	97.40	108.27	106.35	109.19
Mean	92.43	92.39	95.77	106.59	106.86	103.91
S.D.	1.61	1.94	2.19	2.43	2.17	5.38

calibration curves were linear with the coefficient of determinations ranged from 0.9999 to 1.0. All results could be accessible in Appendix C.

2. Release characteristics of ketoprofen rectal suppositories.

2.1 Conventional rectal suppositories.

Conventional hydrophilic rectal suppositories were rapidly released suppositories because ketoprofen was released completely within 30 minutes (Table 11 and Figure 5). The release rate constants of Base 1, Base 2 and Base 3 were 8.21 ± 0.67, 7.26 ± 0.36 and 6.34 ± 0.71 hr^{-1}, respectively (Table 12).

The release of ketoprofen occurred when PEG mixtures dissolved in dissolution medium. The fastest release of ketoprofen was from Base 1, followed by Base 2 and Base 3, respectively. Reasons were due to how fast the base dissolved. Usually, dissolution of PEGs were dependent on their molecular weights. Thus, one with lower molecular weight PEGs could dissolved more rapidly.

For Suppocire® AM, ketoprofen was released only 35.83 % over 24 hours (Table 13 and Figure 6). The release rate constant of Suppocire® AM could not be determined because ketoprofen was not completely released from the base.

Suppocire® AM was hydrophobic suppository base, which melted in dissolution medium. Both ketoprofen and Suppocire® AM were hydrophobic. Binding togethers might be occurred by hydrophobic force. In addition, Suppocire® AM could not dissolve in the buffer dissolution medium. This markedly affected the release of ketoprofen completely from the base. This finding showed that Suppocire ®AM produced prolonged release ketoprofen rectal suppository.

2.2 Matrix rectal suppositories.

Table 11 Percent released of ketoprofen (Mean ± S.D.) from three formulations of conventional hydrophilic ketoprofen rectal suppositories.

Time(min)	% Released		
	Base 1	Base 2	Base 3
2	0.73 ± 0.40	0.77 ± 0.31	0.81 ± 0.72
3	10.26 ± 0.94	8.67 ± 0.64	8.29 ± 1.07
6	23.40 ± 1.22	19.25 ± 1.18	17.91 ± 1.21
8	35.05 ± 1.25	29.53 ± 1.63	27.54 ± 1.62
10	47.06 ± 3.50	38.57 ± 1.67	35.72 ± 1.67
15	67.03 ± 1.36	59.72 ± 3.12	55.48 ± 3.59
20	83.51 ± 1.67	73.99 ± 8.44	73.31 ± 2.91
25	91.90 ± 1.42	88.54 ± 3.74	86.06 ± 3.17
30	93.66 ± 0.97	92.95 ± 2.46	93.12 ± 3.32
35	93.43 ± 1.40	93.73 ± 2.21	94.21 ± 1.51
40	93.64 ± 0.42	93.50 ± 2.75	94.66 ± 1.55
45	93.92 ± 0.95	93.94 ± 1.08	94.82 ± 0.83

n = 6.

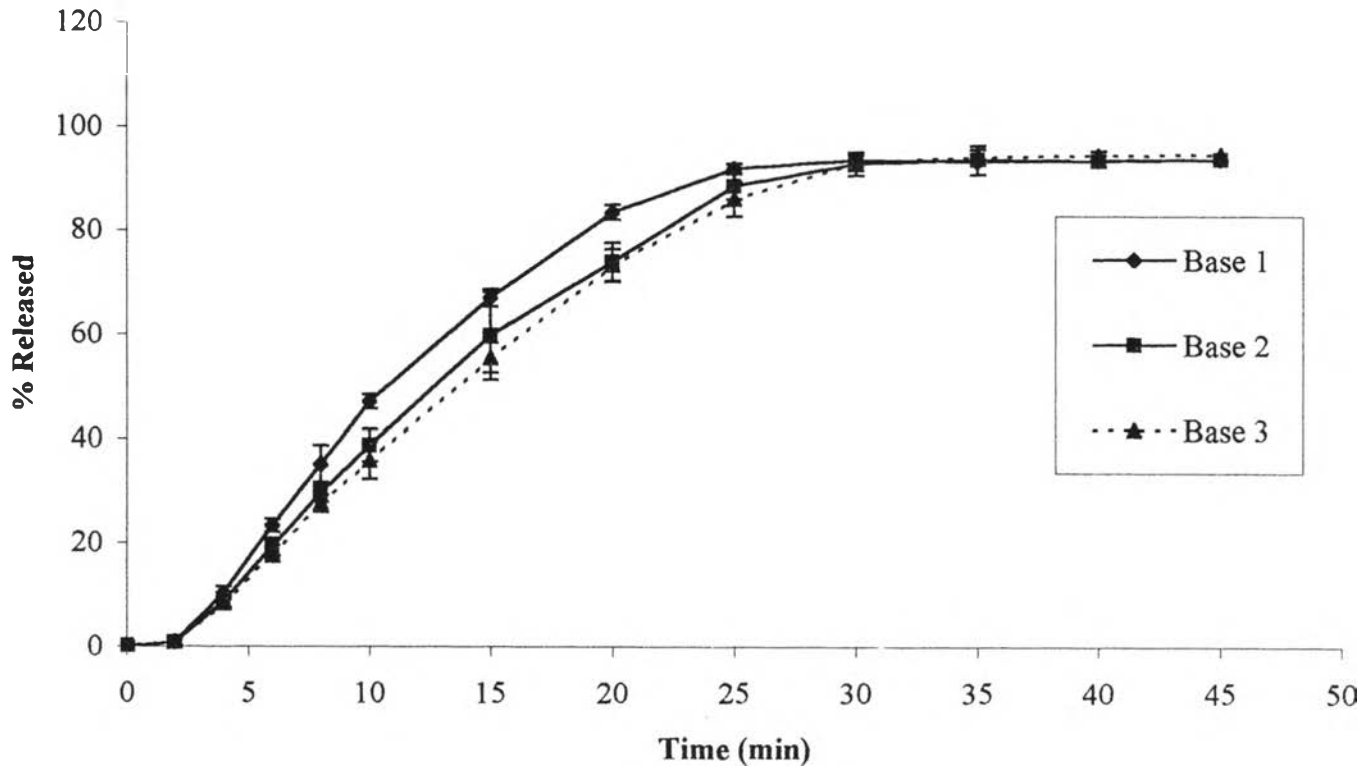


Figure 5 Percent released of ketoprofen from three formulations of conventional hydrophilic ketoprofen rectal suppositories.

Table 12 Release rate constant of ketoprofen (hr^{-1}) from three formulations of conventional ketoprofen rectal suppositories.

Suppositories No.	Release rate constant (hr^{-1})		
	Base 1	Base 2	Base 3
1	8.64	7.08	5.64
2	9.36	7.02	6.06
3	8.04	7.32	7.21
4	7.62	7.86	5.48
5	7.74	7.44	6.64
6	7.86	6.84	6.95
Mean	8.21	7.26	6.34
S.D.	0.67	0.36	0.71

Table 13 Percent released of ketoprofen (Mean ± S.D.) from conventional hydrophobic ketoprofen rectal suppositories.

Time (hr)	% Released
0.08	1.68 ± 0.37
0.17	5.33 ± 0.32
0.25	7.62 ± 0.41
0.5	10.91 ± 0.72
1	15.82 ± 0.93
1.5	17.25 ± 0.94
2	19.17 ± 1.39
3	22.53 ± 1.97
4	24.10 ± 2.26
5	25.55 ± 2.43
6	26.29 ± 2.58
7	27.07 ± 2.61
8	27.97 ± 2.75
9	28.45 ± 2.54
10	28.82 ± 2.21
11	30.09 ± 2.20
12	30.13 ± 2.39
17	32.46 ± 2.06
24	35.83 ± 1.98

n = 6.

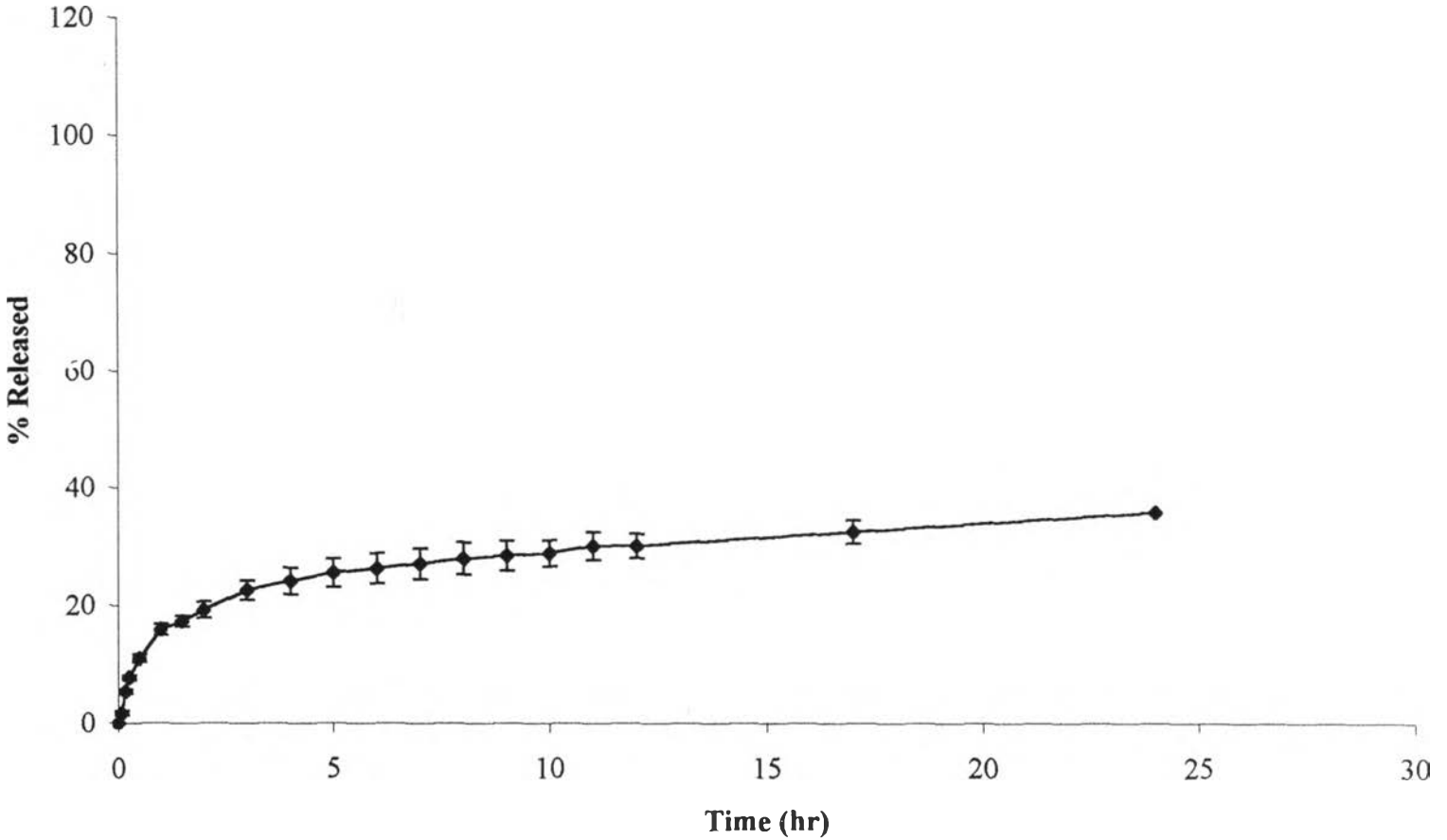


Figure 6 Percent released of ketoprofen from conventional hydrophobic ketoprofen rectal suppostories

The mechanisms of drug release were dissolution of drug from binding with polyethylene glycol on the surface of suppositories where the holes and networks of poorly water soluble carrier occurred. The slow erosion of carrier that formed network made gradually release of the drug (Ohnishi et al. 1987,1988).

2.2.1 Eudragit S-100.

Eudragit S-100 was insoluble in water but gradually dissolved in pH 7.2 buffer solution providing prolonged release of ketoprofen from suppository. The percent released of ketoprofen from suppositories using various ratios of the drug: Eudragit S-100 were shown in Table 14. The release-time profiles were graphically presented in Figure 7-9 and individual release rate constant was reported in Table 15.

The data in Table 14 and their plots of release-time profiles in Figure 7-9 illustrated that all bases of ratio 1:1 and only Base 2 of ratio 1:1.5 could approximately provide complete release of ketoprofen within 4-6 hours without problems. Base 1 of ratio 1:1.5 and two bases (Base 1 and Base 2) of ratio 1:2 required about 7-10 hours. The percent drug released, however, were received about 90 percent. The release patterns of the two of formulations of Base 3 with the ratios of 1:1.5 and 1:2 as seen in Figure 8-9 appeared to be zero-order process. They required more than 10 hours to establish 100 percent released of the drug. The release rate constants computed for all formulations as shown in Table 15 correlated well with the data obtained in Table 14.

2.2.2 Hydroxypropyl methylcellulose phthalate (HP55)

Hydroxypropyl methylcellulose phthalate is widely used in oral pharmaceutical formulations as an enteric coating material. The release rate is pH dependent, HP55 is dissolved in pH 5.5 aqueous buffer solutions (Lachman, Lieberman and Kanig. 1986).

Table 14 Percent released of ketoprofen (Mean ± S.D.) from three formulations of prolonged release ketoprofen rectal suppositories using Eudragit S-100 as prolonged release carrier.

Time	Ketoprofen : Eudragit S-100 = 1:1			Ketoprofen : Eudragit S-100 = 1:1.5			Ketoprofen : Eudragit S-100 = 1:2		
(hr)	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
0.08	2.88 ± 0.17	2.75 ± 0.15	1.25± 0.08	2.62 ± 0.08	2.54 ± 0.34	1.10 ± 0.11	1.27 ± 0.23	0.98 ± 0.25	1.34 ± 0.19
0.17	6.39 ± 0.25	4.40 ± 0.43	2.81 ± 0.20	5.97 ± 0.25	6.84 ± 0.24	2.73 ± 0.12	1.98 ± 0.19	1.27 ± 0.23	1.95 ± 0.09
0.25	9.55 ± 0.35	7.54 ± 0.53	5.39 ± 0.69	9.06 ± 0.32	10.33 ± 0.34	4.39 ± 0.18	5.92 ± 0.26	4.59 ± 0.30	4.03 ± 0.07
0.5	16.88 ± 0.79	14.05 ± 0.71	18.16 ± 2.27	15.29 ± 0.43	20.31 ± 0.62	9.40 ± 0.28	9.46 ± 0.37	7.96 ± 0.45	8.27 ± 0.18
1	31.30 ± 0.95	17.60 ± 1.00	35.84 ± 4.32	25.56 ± 0.81	30.76 ± 1.19	18.56 ± 1.17	15.02 ± 0.39	15.59 ± 0.56	14.34 ± 0.24
1.5	42.16 ± 1.23	39.99 ± 1.40	51.67 ± 5.33	32.55 ± 1.04	40.90 ± 1.20	22.29 ± 1.99	24.51 ± 0.57	24.01 ± 0.10	16.91 ± 0.27
2	51.06 ± 1.50	50.48 ± 2.48	64.76 ± 5.92	40.18 ± 1.44	49.75 ± 1.54	27.41 ± 2.57	32.36 ± 0.97	31.36 ± 0.86	19.79 ± 0.62
3	66.74 ± 2.06	68.37 ± 3.39	84.74 ± 8.31	50.79 ± 0.94	65.40 ± 1.82	35.00 ± 3.82	39.31 ± 1.08	38.23 ± 0.97	26.63 ± 0.51
4	76.52 ± 2.92	80.20 ± 3.82	100.34 ± 4.28	59.56 ± 2.10	79.28 ± 1.93	42.84 ± 5.83	52.58 ± 1.97	50.81 ± 1.26	31.14 ± 1.07
5	91.04 ± 4.02	92.18 ± 3.57	99.79 ± 2.96	66.71 ± 2.20	91.47 ± 1.71	49.47 ± 8.20	63.12 ± 0.92	59.98 ± 1.49	35.73 ± 0.88
6	97.42 ± 1.56	96.44 ± 3.96	102.26 ± 2.82	73.90 ± 4.14	101.05 ± 1.31	54.95 ± 8.74	72.11 ± 1.18	69.04 ± 0.88	40.51 ± 0.90
7	98.13 ± 2.18	95.83 ± 2.79	101.61 ± 2.39	81.97 ± 3.77	106.80 ± 2.08	61.17 ± 11.01	79.32 ± 1.36	75.50 ± 1.69	44.41 ± 0.94
8	97.27 ± 1.50	96.04 ± 3.11	100.60 ± 2.52	86.95 ± 3.16	108.35 ± 1.14	68.92 ± 12.75	84.51 ± 2.01	80.60 ± 2.02	47.98 ± 1.37
9	97.52 ± 1.26	94.42 ± 3.45	100.95 ± 2.12	90.33 ± 1.12	108.57 ± 0.95	76.33 ± 12.75	89.25 ± 2.63	88.09 ± 2.50	50.46 ± 3.78
10	96.79 ± 1.25	94.58 ± 3.94	100.82 ± 1.75	90.98 ± 0.68	107.82 ± 0.10	81.15 ± 10.60	93.00 ± 1.81	90.79 ± 1.62	58.12 ± 3.50

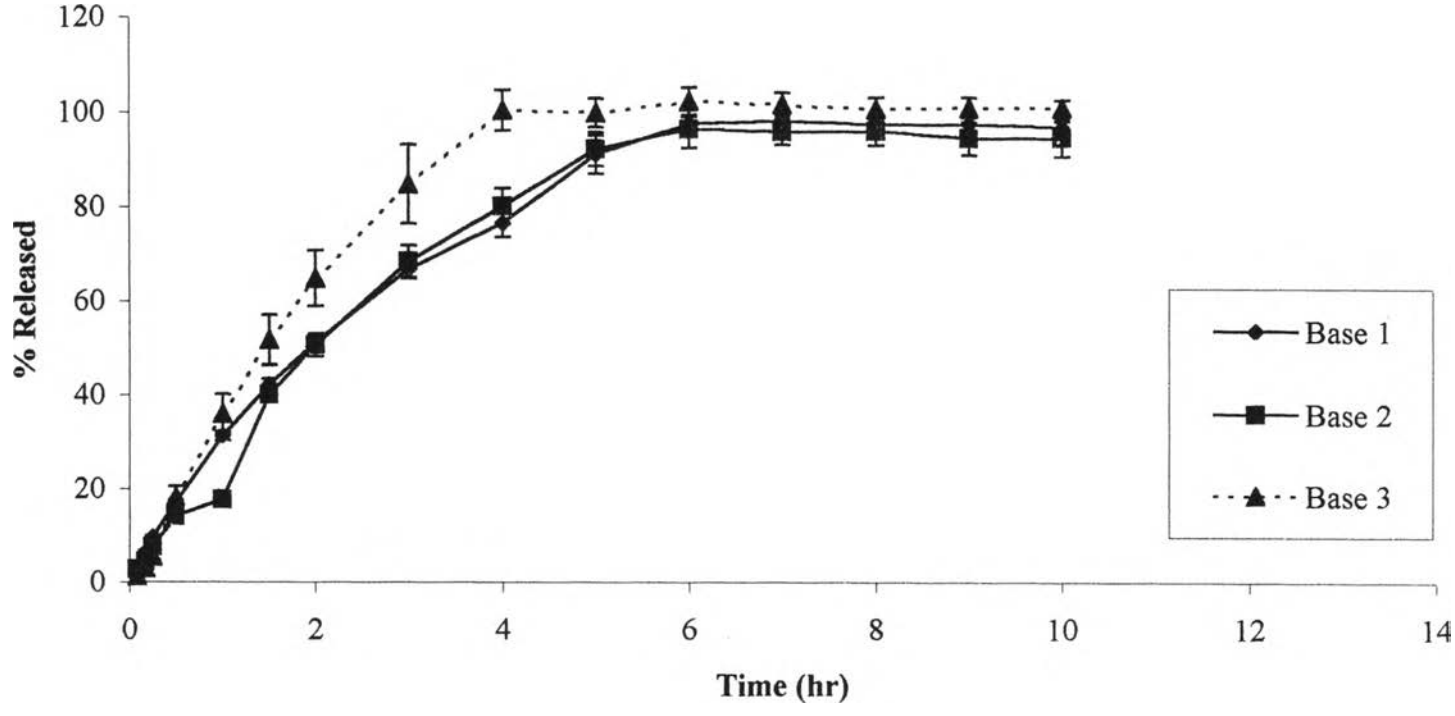


Figure 7 Percent released of ketoprofen from three formulations of prolonged release ketoprofen rectal suppositories using ketoprofen : Eudragit S-100 = 1:1.

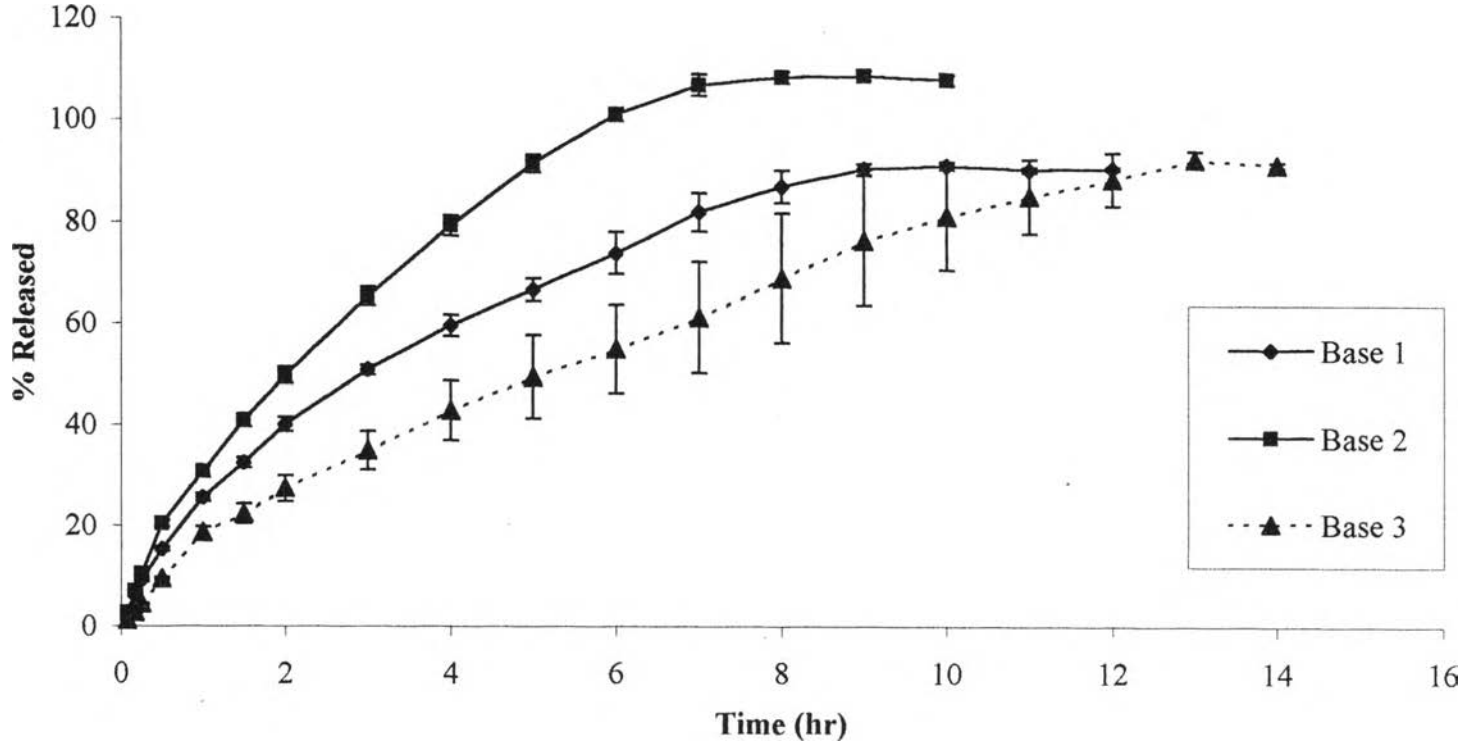


Figure 8 Percent released of ketoprofen from three formulations of prolonged release ketoprofen rectal suppositories using ketoprofen : Eudragit S-100 = 1:1.5.

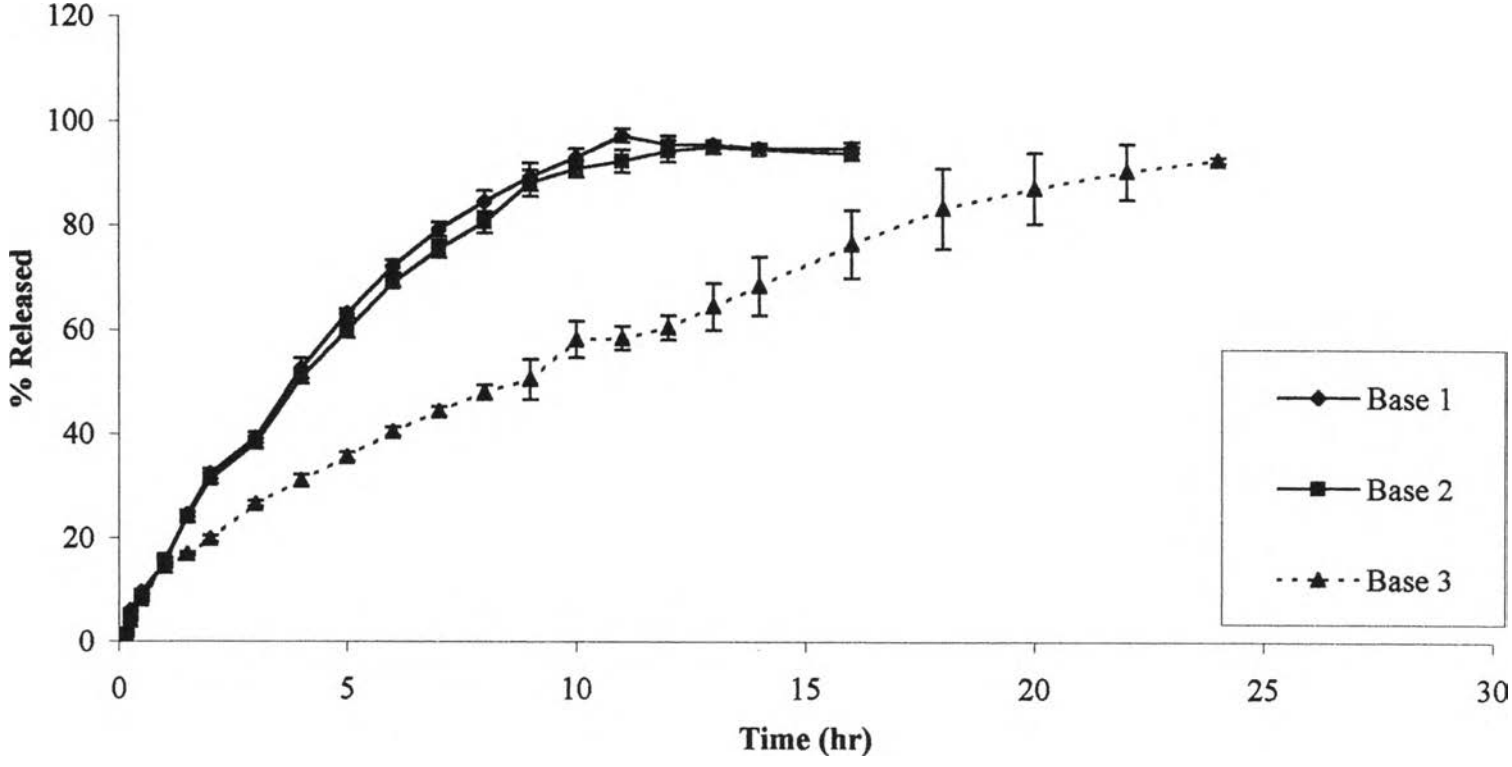


Figure 9 Percent released of ketoprofen from three formulations of prolonged release ketoprofen rectal suppositories using ketoprofen : Eudragit S-100 = 1:2.

Table 15 Release rate constant of ketoprofen (hr^{-1}) from three formulations of prolonged release ketoprofen rectal suppositories using Eudragit S-100 as prolonged release carrier.

Suppositories	Ketoprofen : Eudragit S-100 = 1:1			Ketoprofen : Eudragit S-100 = 1:1.5			Ketoprofen : Eudragit S-100 = 1:2		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	0.41	0.50	0.61	0.36	0.43	0.16	0.38	0.31	0.10
2	0.55	0.47	0.71	0.34	0.44	0.29	0.32	0.31	0.08
3	0.46	0.47	0.58	0.29	0.44	0.23	0.34	0.33	0.16
4	0.36	0.52	0.51	0.29	0.46	0.22	0.30	0.32	0.12
5	0.42	0.40	0.60	0.27	0.27	0.26	0.38	0.37	0.10
6	0.46	0.55	0.58	0.35	0.40	0.14	0.34	0.32	0.11
Mean	0.44	0.49	0.60	0.32	0.41	0.22	0.34	0.33	0.11
S.D.	0.06	0.05	0.06	0.04	0.07	0.06	0.04	0.03	0.03

The percent released of ketoprofen from suppositories prepared using HP55 as prolonged release carrier in hydrophilic bases for all ratios between the drug to HP55 were presented in Table 16 and Figure 10-11. The release rate constants of all formulations were presented in Table 17. Results demonstrated that all bases of both ratios except Base 3 of ratio 1:4 exhibited completely release of the drug within 3 hours. The one with exception required 4 hours to produce such percent released of ketoprofen. The release rate constants in Table 17 of each formulation revealed to be relative with the corresponding data.

2.3 Selection of the best formulation.

All data for *in vitro* studies were summarized in Table 18. Two formulations of 100 mg prolonged release ketoprofen rectal suppositories prepared using hydrophilic bases were selected according to the criteria specified earlier. One was the formula with specific ratio of ketoprofen to Eudragit S-100 and another was the preparation with that of ketoprofen : HP55.

For Eudragit S-100, all formulations of ratio 1:1 and one formulation (Base 2) of ratio 1:1.5 provided equally results. However, Base 2 of ratio 1:1.5 was deleted according to use larger amount of carrier. Consideration all the rest, Base 1 could be selected based on lowest release rate constant.

In the case of HP55, the ratio of 1:4 was preferable to that of 1:3 based on the data in Table 16. Among all bases of ratio 1:4, Base 3 was selected due to its slowest release rate constant.

3. *In Vivo* studied.

100 mg prolonged release ketoprofen rectal suppositories using Base 1 of ketoprofen : Eudragit S-100 = 1:1, Base 3 of ketoprofen : HP55 = 1:4 and Suppocire® AM were freshly prepared to be used for *in vivo* studies. They were tested and conformed for uniformity of weight and uniformity of content of BP 1993.

Table 16 Percent released of ketoprofen (Mean ± S.D.) from two formulations of prolonged release ketoprofen rectal suppositories using HP55 as prolonged release carrier.

Time	Ketoprofen : HP55 = 1:3			Ketoprofen : HP55 = 1:4		
(hr)	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
0.08	1.59 ± 0.21	0.72 ± 0.15	0.61 ± 0.17	1.38 ± 0.20	1.03 ± 0.25	0.85 ± 0.16
0.17	4.63 ± 0.32	4.31 + 0.40	2.50 ± 0.47	3.97 ± 0.17	3.97 ± 0.35	2.17 ± 0.36
0.25	7.75 ± 0.59	8.76 ± 2.58	4.61 ± 0.81	8.34 ± 0.31	8.02 ± 0.53	3.76 ± 0.49
0.5	18.94 ± 1.58	14.55 ± 2.40	11.17 ± 1.70	23.02 ± 8.79	19.01 ± 1.26	10.36 ± 0.92
1	37.58 ± 1.32	37.29 ± 1.74	26.71 ± 4.05	41.37 ± 1.53	40.01 ± 1.90	23.31 ± 1.47
1.5	56.22 ± 2.03	54.50 ± 6.53	46.79 ± 7.94	60.30 ± 2.48	58.83 ± 2.63	39.42 ± 1.57
2	71.28 ± 2.76	71.91 ± 2.37	67.17 ± 8.63	73.66 ± 3.54	78.19 ± 3.99	55.47 ± 2.95
3	90.93 ± 2.62	96.20 ± 2.37	91.01 ± 2.52	101.34 ± 6.96	103.45 ± 5.05	85.66 ± 2.82
4	94.05 ± 1.32	97.39 ± 2.13	95.01 ± 3.88	107.45 ± 3.55	106.30 ± 3.89	103.61 ± 1.51
5	93.91 ± 0.65	96.65 ± 1.81	95.38 ± 3.45	108.54 ± 3.67	105.85 ± 2.99	106.41 ± 1.72
6	-	-	-	108.62 ± 3.64	106.52 ± 3.13	107.39 ± 1.25

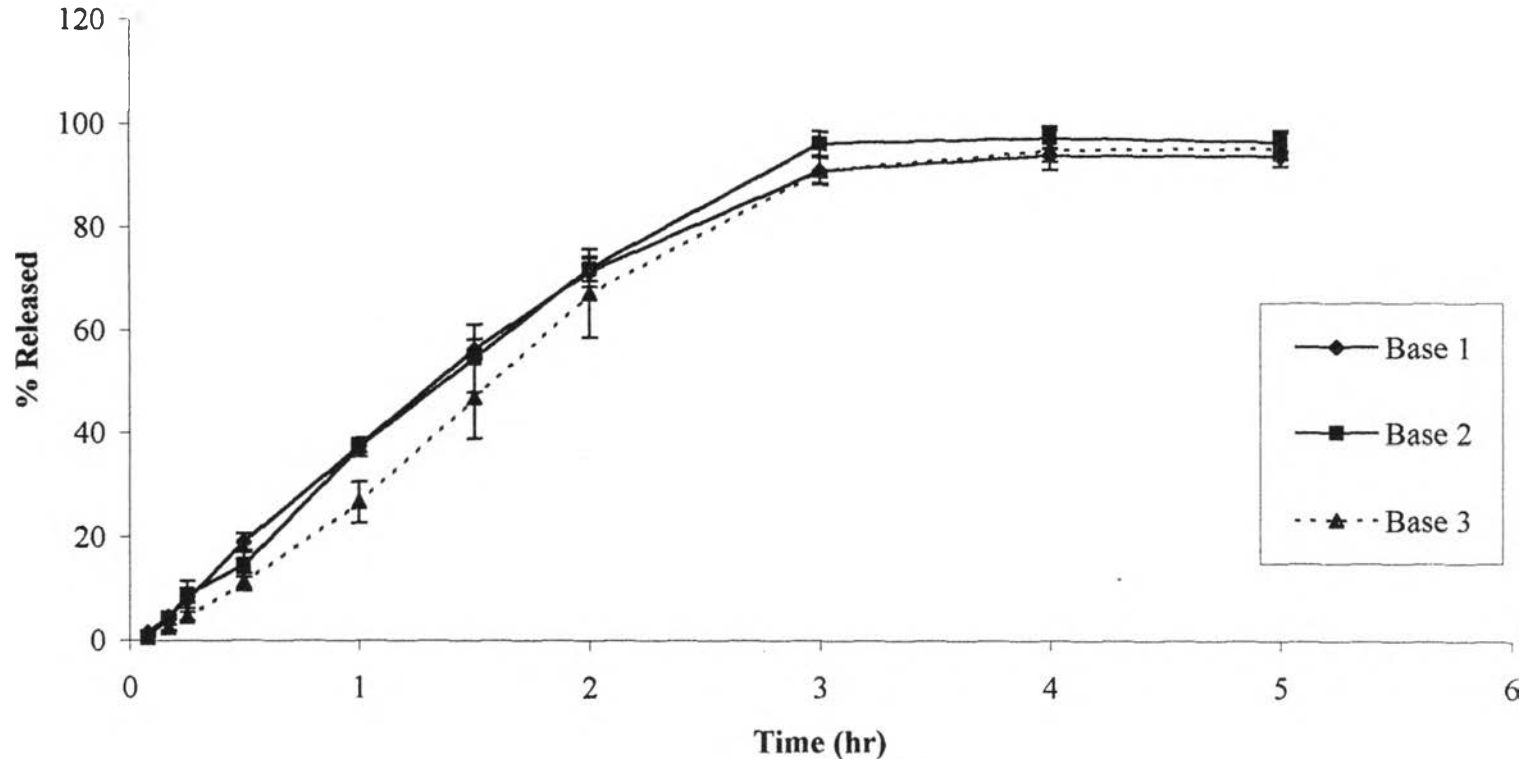


Figure 10 Percent released of ketoprofen from three formulations of prolonged release ketoprofen rectal suppositories using ketoprofen : HP55 = 1:3.

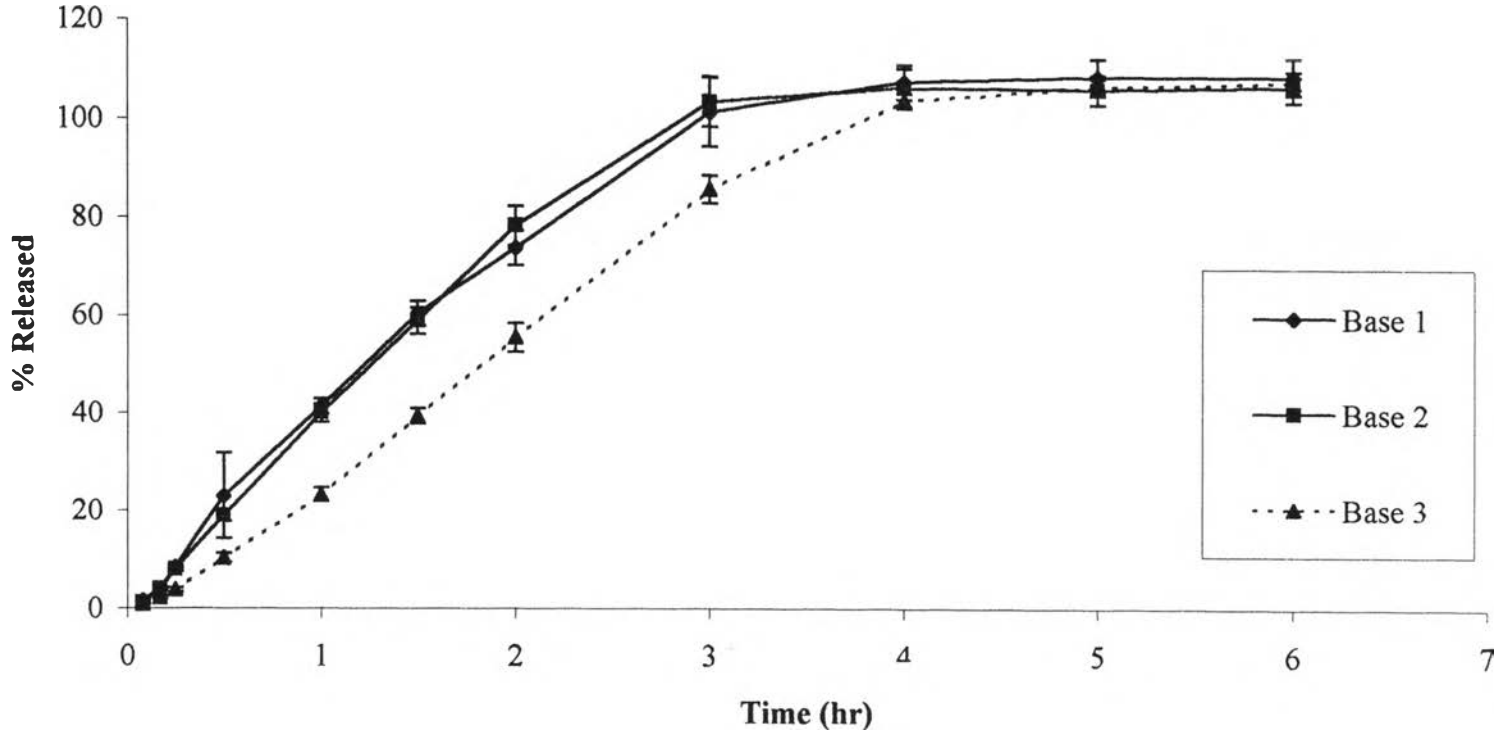


Figure 11 Percent released of ketoprofen from three formulations of prolonged release ketoprofen rectal suppositories using ketoprofen : HP55 = 1:4.

Table 17 Release rate constant of ketoprofen (hr^{-1}) from two formulations of prolonged release ketoprofen rectal suppositories using HP55 as prolonged release carrier.

Suppositories	Ketoprofen : HP55 = 1:3			Ketoprofen : HP55 = 1:4		
No.	Base 1	Base 2	Base 3	Base 1	Base 2	Base 3
1	0.80	0.62	0.68	0.71	0.67	0.54
2	0.87	0.71	0.61	0.75	0.69	0.55
3	0.80	0.76	1.18	0.79	0.67	0.58
4	0.70	0.69	0.76	0.80	0.71	0.50
5	0.71	0.70	0.63	0.55	0.71	0.61
6	0.68	0.64	0.68	0.70	0.69	0.55
Mean	0.76	0.69	0.76	0.72	0.69	0.56
S.D.	0.08	0.05	0.22	0.09	0.02	0.04

Table 18 Summary of *in vitro* studies of all formulations of ketoprofen rectal suppositories.

Formulations	Uniformity of weight (g)				Uniformity of content (%L.A.)				Release rate constant (hr^{-1})			
	Base 1	Base 2	Base 3	Suppocire ®AM	Base 1	Base 2	Base 3	Suppocire ®AM	Base 1	Base 2	Base 3	Suppocire ®AM
1. Conventional rectal suppositories	2.4450±0.01	2.4508±0.01	2.5095±0.20	1.9677±0.01	93.54±1.07	94.59±1.72	92.33±1.70	97.20±3.63	8.21±0.67	7.26±0.36	6.34±0.71	-
2. Matrix rectal suppositories												
2.1 Ketoprofen / Eudragit S-100 ratios												
1 : 1	2.4505±0.02	2.4450±0.02	2.5065±0.03	-	99.21±3.17	103.10±4.24	98.51±3.82	-	0.44±0.06	0.49±0.05	0.60±0.06	-
1 : 1.5	2.4354±0.03	2.4187±0.03	2.4632±0.03	-	91.86±1.43	100.23±4.17	92.65±1.94	-	0.32±0.04	0.41±0.07	0.22±0.06	-
1 : 2	2.4748±0.01	2.4517±0.01	2.4553±0.03	-	96.32±4.11	98.98±6.29	92.90±1.53	-	0.34±0.04	0.33±0.03	0.11±0.03	-
2.2 Ketoprofen / HP55 ratios												
1 : 3	2.4997±0.03	2.4976±0.03	2.4965±0.03	-	92.43±1.61	92.39±1.94	95.77±2.19	-	0.76±0.08	0.69±0.05	0.76±0.22	-
1 : 4	2.5091±0.01	2.4594±0.03	2.4600±0.02	-	106.59±2.43	106.86±2.17	103.91±5.38	-	0.72±0.09	0.69±0.02	0.56±0.04	

3.1 Analysis of ketoprofen concentrations in rabbit plasma.

Figure 12 showed chromatograms of blank rabbit plasma, rabbit plasma spiked with ketoprofen and internal standard, and plasma sample taken at 8 hours postdose from a rabbit following administration of 100 mg prolonged release ketoprofen rectal suppository. Ketoprofen and diclofenac sodium was well separated from the endogenous substance peak with the retention times of 5.65 and 8.17 min, respectively.

The validation of analytical method for determination of ketoprofen in rabbit plasma was performed by determining the accuracy, the within run and between run precisions. Results were accessible in Appendix E. The accuracy in term of percent recovery at all concentrations was between 95.02-103.83 %. The within run and between run precisions expressed as percent coefficient of variations were 2.01-5.48% and 1.33-10.98%, respectively. The calibration curve of PAR of ketoprofen to diclofenac sodium versus plasma ketoprofen concentrations was linear covered all concentration used with the coefficient of determination of 0.9995.

3.2 Plasma ketoprofen concentrations.

Plasma ketoprofen concentrations at any sampling time interval up to 24 hours from 9 nine rabbits after rectal administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories were presented in Table 19 to 21. Comparisons of the plasma ketoprofen concentration-time profiles of each rabbit were illustrated in Figures 13 to 22 and all profiles were summarized for 9 rabbits graphically in Figure 23. Although the plasma ketoprofen concentrations versus time profiles appeared to be similar to those with immediately release formulations, these patterns were judged to be prolonged release. Evidence was supported by the bases used in this study has been proven to produce prolonged release of hydrophobic drugs from suppositories (Ohnishi et al. 1986, 1988; Ermis and Tarimci, 1995). Moreover, ketoprofen could be detected in rabbit plasma samples up to 24 hours, referring prolonged release of the formulated suppositories. Most of individual profiles were

smooth except those of the rabbit no. 1 after receiving the formula with Eudragit S-100 and the rabbit no.6 after administering the suppositories with HP55 exhibited reversed concentrations at the time points between 0.5 and 1.0 hours. This was attributed by experimental errors.

3.3 Pharmacokinetics studies.

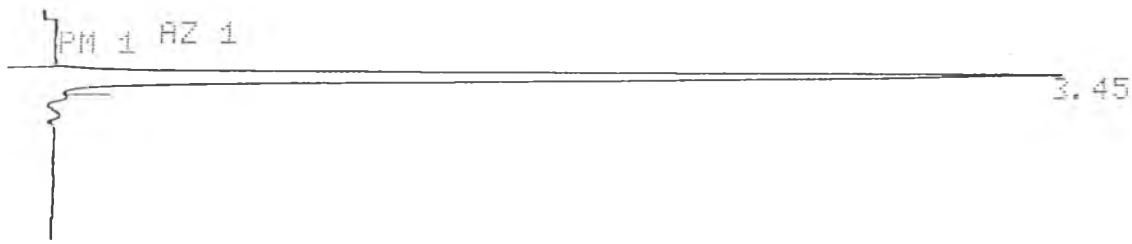
The pharmacokinetics of ketoprofen following administration of all formulations of 100 mg prolonged release of ketoprofen rectal suppositories in 9 rabbits appeared to be multicompartment models. This was evident by, after the peak concentration of the drug was attained, the concentrations of ketoprofen declined rapidly and then more slowly afterward (Gibaldi and Perrierr. 1982). Since the number of subjects included in this study were quite small, analysis of the plasma ketoprofen concentrations-time curves for the relevant pharmacokinetic parameters especially the peak plasma ketoprofen concentrations and the area under the plasma ketoprofen concentrations-time profile in terms of log transformed data to be used for bioavailability comparisons were preferable to using normal data (US FDA. 1992).

The relevant pharmacokinetic parameters obtained for bioavailability comparison were reported as follows:

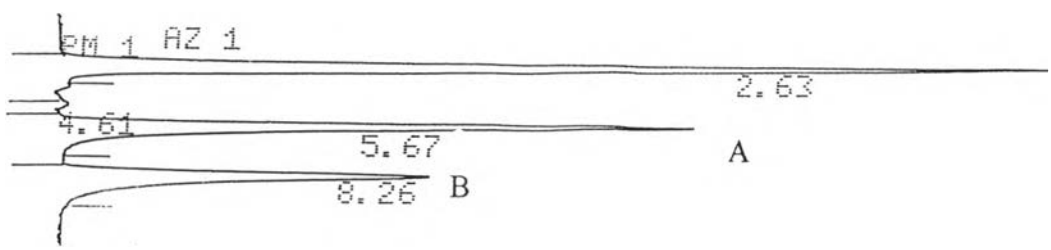
Peak plasma ketoprofen concentration (C_{max})

The log C_{max} (Mean ± S.D.) of ketoprofen obtained from suppositories with Eudragit S-100, HP55 and Suppocire ®AM were 2.12 ± 0.33, 2.11 ± 0.21 and 2.19 ± 0.35 μg/mL (Table 22), respectively. Statistical comparison in Table 23 showed that all three formulations were not statistically significant differences ($p > 0.05$) from each other.

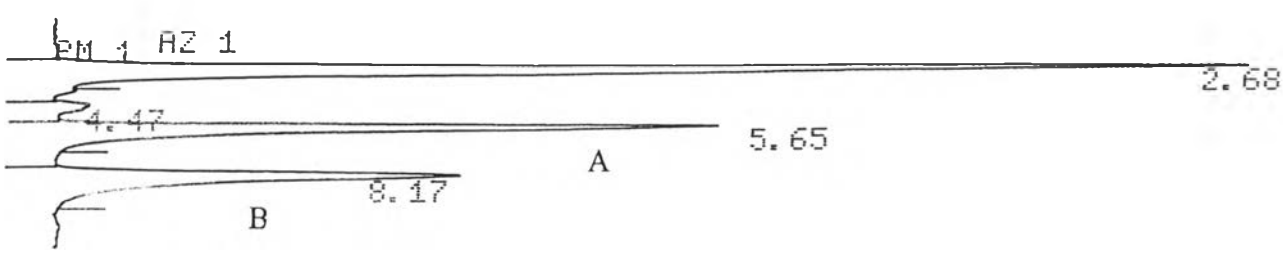
Time to peak plasma ketoprofen concentration (t_{max})



blank rabbit plasma



Spiked rabbit plasma (ketoprofen conc. 20 μg/mL)



Rabbit plasma sample taken at 8 hours postdose (ketoprofen observed conc. 17.27 μg/mL)

Figure 12 High performance liquid chromatograms of ketoprofen (A) and diclofenac sodium (B).

Table 19 Plasma ketoprofen concentration (μg/mL) of nine rabbits after administration of 100 mg prolonged release ketoprofen rectal suppositories using Eudragit S-100 as prolonged release carrier.

Rabbit No.	Time (hr)										
	0.5	1	2	3	4	6	8	10	14	18	24
1	38.96	29.96	101.33	79.65	62.16	34.71	18.49	10.68	4.13	2.50	0.93
2	207.56	188.61	134.42	99.21	66.21	35.72	17.39	6.69	0.98	0.99	0.70
3	118.42	134.23	127.30	109.59	64.07	40.23	22.93	11.12	8.12	1.55	0.73
4	155.65	177.58	127.49	88.22	67.30	42.54	24.95	15.10	3.71	0.81	0.64
5	152.43	150.77	101.58	68.69	49.11	27.58	13.23	5.33	2.12	1.32	0.56
6	115.70	156.53	110.81	77.07	64.22	36.06	22.18	13.26	3.45	1.02	1.17
7	32.18	22.79	22.32	15.56	12.46	7.60	5.22	4.48	3.10	3.04	1.69
8	155.15	141.53	91.39	78.24	58.45	29.86	15.74	7.00	2.34	1.44	0.80
9	204.73	166.10	124.42	91.84	72.99	46.43	32.72	20.93	7.94	2.34	0.97
Mean	131.20	129.79	104.56	78.67	57.44	35.41	19.20	10.51	3.99	1.67	0.91
S.D.	62.83	61.02	34.14	26.76	18.10	11.33	7.79	5.34	2.48	0.78	0.35

Table 20 Plasma ketoprofen concentration (μg/mL) of nine rabbits after administration of 100 mg prolonged release ketoprofen rectal suppositories using HP55 as prolonged release carrier.

Rabbit No.	Time (hr)										
	0.5	1	2	3	4	6	8	10	14	18	24
1	119.13	129.23	85.19	57.17	42.46	22.92	14.12	7.17	3.17	1.51	0.69
2	205.53	226.48	117.26	87.28	66.34	42.00	23.94	17.38	7.53	2.23	1.22
3	95.49	93.69	85.77	69.51	55.17	33.97	20.62	10.10	3.77	0.78	0.57
4	155.17	137.46	111.05	83.13	61.28	39.81	24.58	12.25	4.43	1.75	2.86
5	138.85	129.99	85.03	59.48	44.74	44.55	29.15	15.76	3.37	1.85	0.00
6	107.12	128.03	77.28	61.72	41.03	24.23	13.49	7.62	2.79	3.04	0.75
7	15.48	56.72	58.41	61.76	50.64	24.39	14.77	8.46	1.31	1.17	0.69
8	116.17	144.94	118.64	89.46	58.69	33.32	16.28	6.69	3.31	2.84	1.04
9	138.44	148.46	103.48	79.18	59.34	36.27	26.62	18.55	9.74	7.54	10.93
Mean	121.26	132.78	93.57	72.08	53.30	33.50	20.40	11.55	4.38	2.52	2.08
S.D.	51.16	45.48	20.31	12.78	9.02	8.09	5.93	4.63	2.61	2.02	3.41

Table 21 Plasma ketoprofen concentration (μg/mL) of nine rabbits after administration of 100 mg prolonged release ketoprofen rectal suppositories using Suppocire® AM as base.

Rabbit No.	Time (hr)										
	0.5	1	2	3	4	6	8	10	14	18	24
1	114.81	91.14	77.49	62.11	56.57	36.27	22.36	20.75	9.81	7.70	5.20
2	244.79	219.16	216.34	140.13	98.62	57.43	26.05	13.19	2.17	2.26	0.78
3	263.94	248.77	177.42	150.81	126.20	90.27	51.58	33.36	11.23	2.91	0.94
4	207.04	159.62	122.42	90.47	61.55	31.12	13.61	5.83	2.47	1.84	0.80
5	215.71	143.68	79.23	75.56	45.18	22.64	5.58	4.77	1.64	1.03	0.74
6	14.98	9.54	29.70	32.52	36.05	33.07	27.24	24.38	14.03	5.82	2.72
7	100.06	119.57	134.80	97.25	76.15	55.58	34.72	25.78	14.27	9.36	5.19
8	160.64	179.41	143.51	102.94	81.63	44.09	26.22	12.19	1.46	1.22	0.72
9	132.07	152.66	160.98	112.35	86.87	55.40	36.85	23.49	10.51	3.80	0.71
Mean	161.56	147.06	126.88	96.01	74.31	47.32	27.13	18.19	7.51	3.99	1.98
S.D.	79.64	70.34	57.26	36.90	28.04	20.27	13.33	9.71	5.49	2.99	1.93

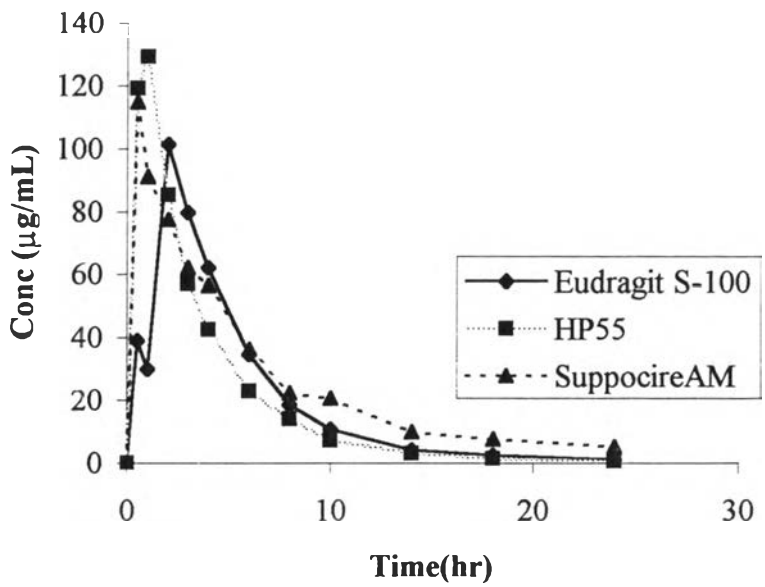


Figure 13 Plasma ketoprofen concentration-time curves of rabbit No.1 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

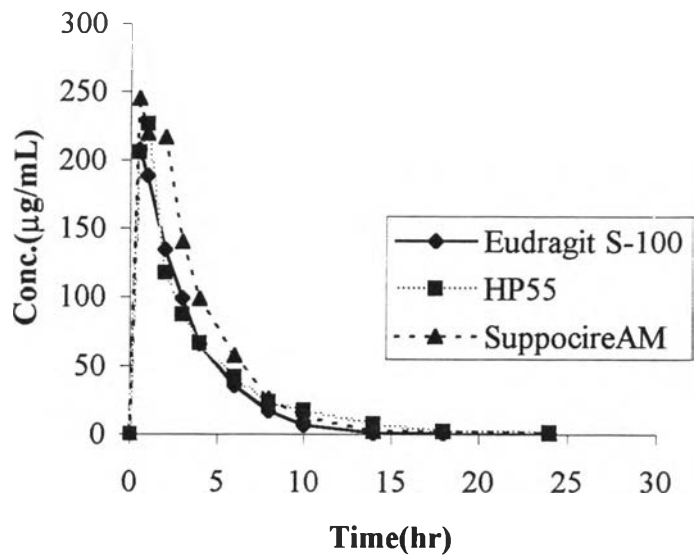


Figure 14 Plasma ketoprofen concentration-time curves of rabbit No.2 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

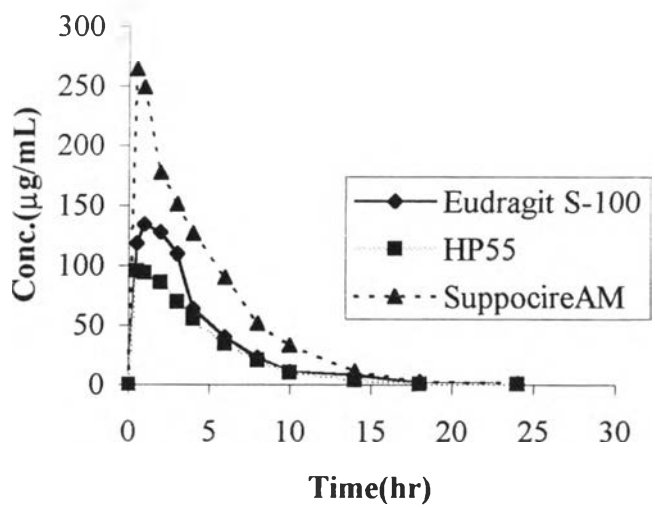


Figure 15 Plasma ketoprofen concentration-time curves of rabbit No.3 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

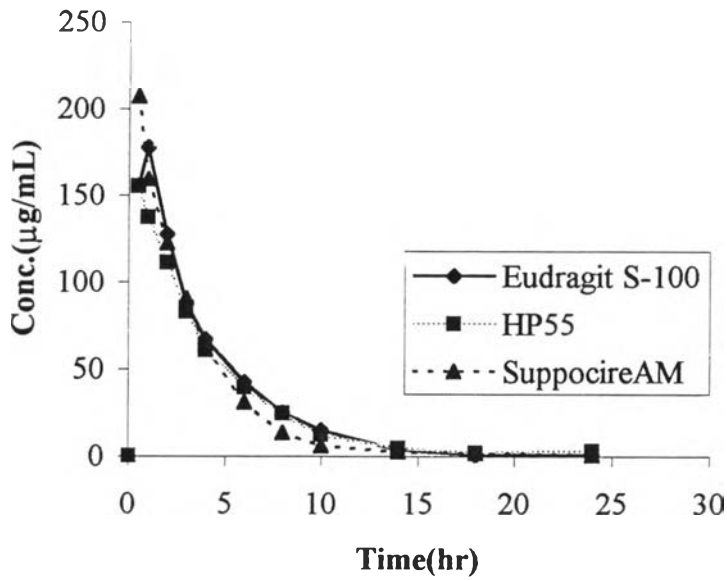


Figure 16 Plasma ketoprofen concentration-time curves of rabbit No.4 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

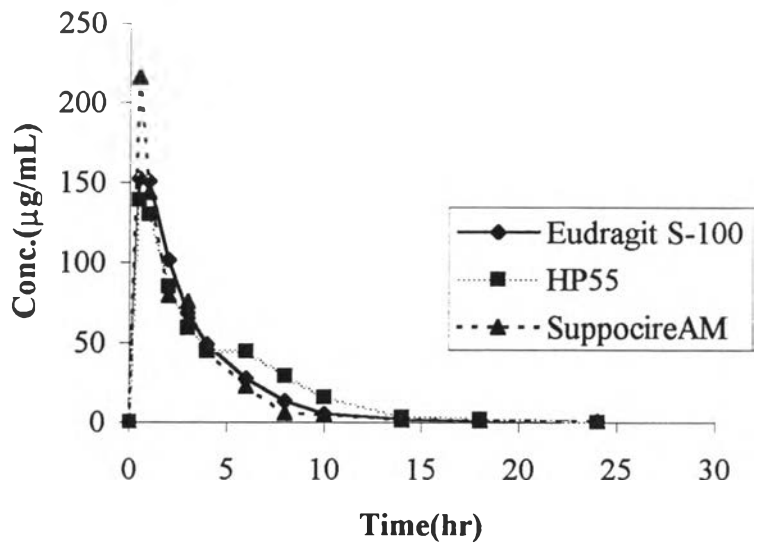


Figure 17 Plasma ketoprofen concentration-time curves of rabbit No.5 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

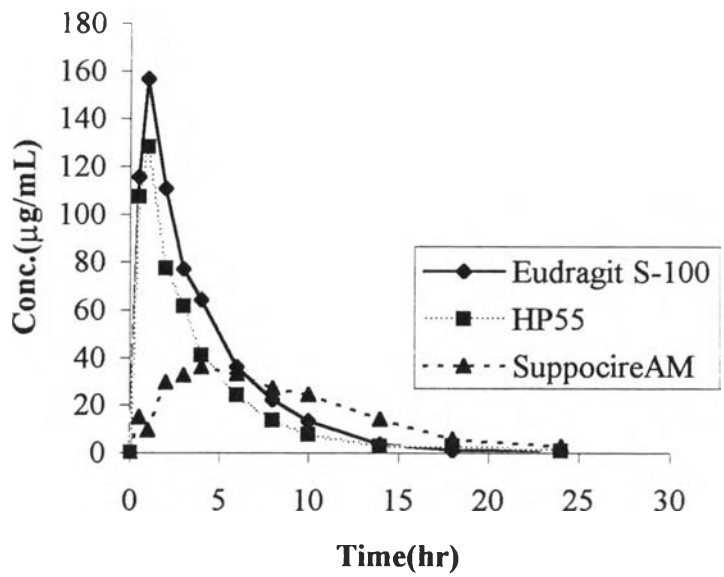


Figure 18 Plasma ketoprofen concentration-time curves of rabbit No.6 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

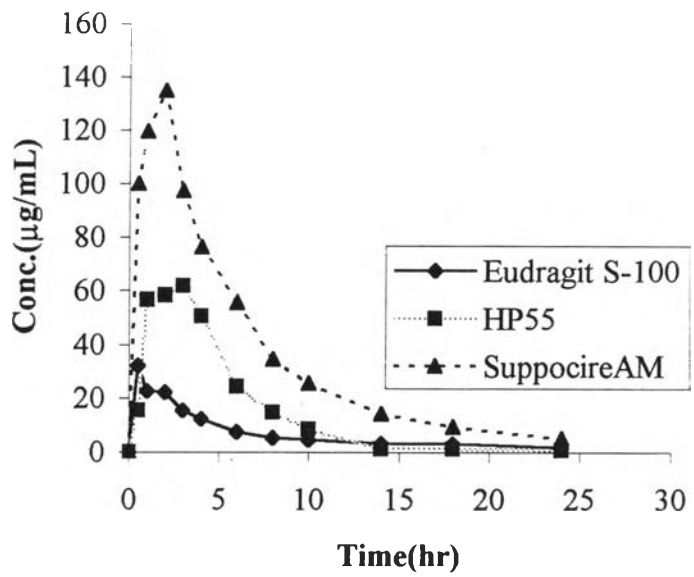


Figure 19 Plasma ketoprofen concentration-time curves of rabbit No.7 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

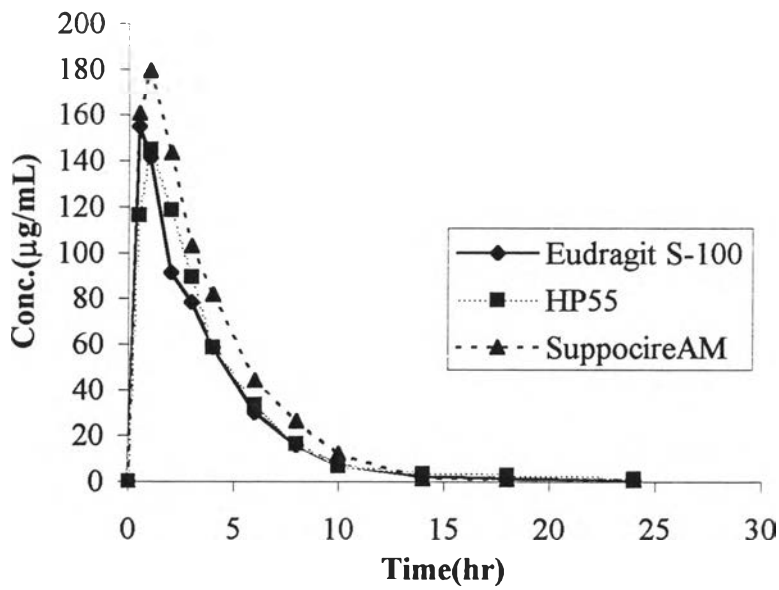


Figure 20 Plasma ketoprofen concentration-time curves of rabbit No.8 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

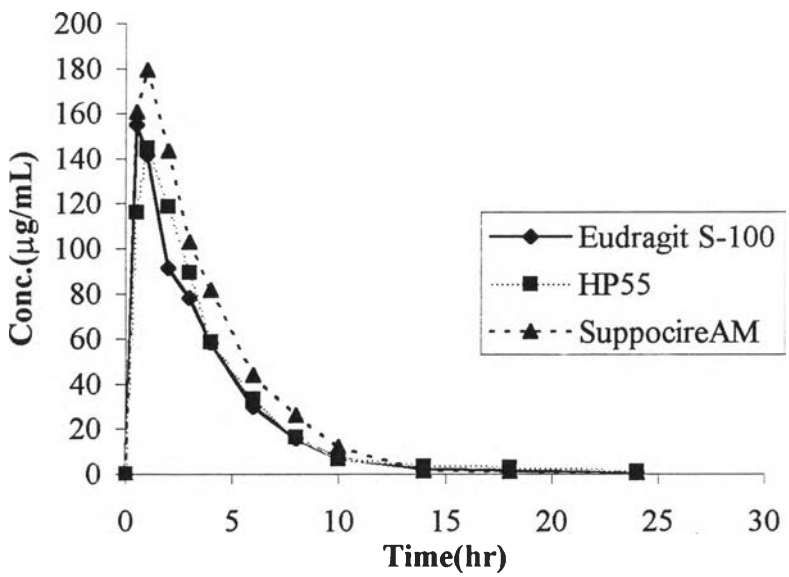


Figure 21 Plasma ketoprofen concentration-time curves of rabbit No.9 after administration of three formulations of prolonged release ketoprofen rectal suppositories.

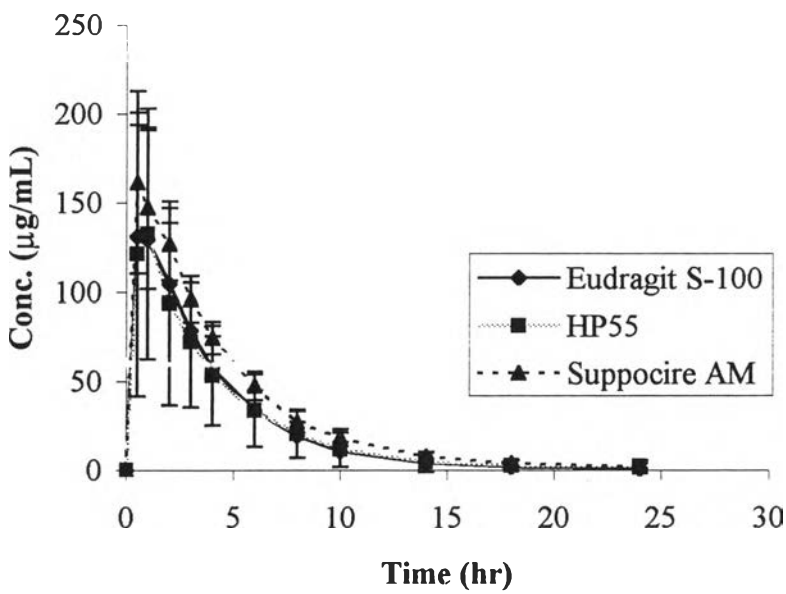


Figure 22 Comparison of plasma ketoprofen concentration-time curves of nine rabbits after administration of three formulations of prolonged release ketoprofen rectal suppositories.

Table 22 Log of peak plasma ketoprofen concentrations (log C_{max}) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	log C_{max} (μg/mL)		
	Eudragit S-100	HP55	Suppocire ®AM
1	2.01	2.11	2.06
2	2.32	2.36	2.39
3	2.13	1.98	2.42
4	2.25	2.19	2.32
5	2.18	2.14	2.33
6	2.19	2.11	1.56
7	1.51	1.79	2.13
8	2.19	2.16	2.25
9	2.31	2.17	2.21
Mean	2.12	2.11	2.19
S.D.	0.33	0.21	0.35

Table 23 Analysis of variance for three way crossover design of log C_{max} of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05) and 90 percent confidence interval for the difference of C_{max} means.

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	1.26	0.05	-	-	-
Sequence	2	0.06	0.03	0.27	5.14	NS
Subject (Sequence)	6	0.68	0.11	1.95	2.64	NS
Period	2	0.04	0.02	0.35	3.68	NS
Formulation	2	0.03	0.02	0.26	3.68	NS
Error	14	0.81	0.06	-	-	-

1. 90% CI of suppository with Eudragit S-100 = 87.67 – 105.94
2. 90% CI of suppository with HP55 = 87.21 – 105.48

How fast of drug absorption was indicated by this parameter. All formulations were rapidly absorbed as seen by the peak plasma concentration was reached to a maximum value in a short period of time, as shown in Table 24. The t_{max} (Mean ± S.D.) of ketoprofen from suppositories with Eudragit S-100, HP55 and Suppocire® AM were 0.83 ± 0.50, 1.06 ± 0.77 and 1.28 ± 1.20 hours, respectively. There were no statistically significant differences ($p>0.05$) among t_{max} values of these three formulations (Table 25).

Area under the plasma ketoprofen concentration-time curve (AUC)

This parameters represented the extent or total amount of ketoprofen absorption into the systemic circulation. The log AUC (Mean ± S.D.) from suppositories with Eudragit S-100, HP55 and Suppocire ®AM were 2.75 ± 0.27, 2.76 ± 0.16 and 2.88 ± 0.20 μg.hr/mL (Table 26). Statistical comparison in Table 27 showed that all three formulations were not statistically significant differences ($p> 0.05$).

Other pharmacokinetic parameters of ketoprofen established in this study were described as follows:

Elimination rate constant (K_{el})

The elimination rate constant was the slope of the terminal phase determined by linear regression analysis of the plot of natural logarithm of plasma concentration-time profile. Table 28 showed the average (Mean ± S.D.) elimination rate constants of the products with Eudragit S-100, HP55 and Suppocire ®AM which were 0.24 ± 0.06, 0.22 ± 0.05 and 0.21 ± 0.08 $hour^{-1}$, respectively. Statistical comparison in Table 29 revealed that all three formulations were not statistically significant differences ($p>0.05$).

Elimination half-life ($t_{1/2}$)

The average (Mean ± S.D.) elimination half-lives of suppositories prepared with Eudragit S-100, HP55 and Suppocire ®AM were 3.23 ± 1.46, 3.31 ± 1.31 and 3.64 ± 2.04 hour, respectively (Table 30). Statistical comparison in Table 31 indicated that all three formulations were not statistically significant differences ($p > 0.05$).

Mean residence time (MRT)

Mean residence time was the time for 63.2% of the administered dose to be eliminated. The more value of mean residence time was, the longer time of drug residing in systemic circulation was observed. Therefore the pharmacological response intended to be more effective. Following Table 32 the average (Mean ± S.D.) mean residence time of the formulations with Eudragit S-100, HP55 and Suppocire ® AM were 4.65 ± 2.11, 4.78 ± 1.89 and 5.25 ± 2.95 hour, respectively. Statistical comparison in Table 33 presented that all three formulations were not statistically significant differences ($p > 0.05$).

Volume of distribution (V_d/F)

In this study, the fraction of drug absorbed (F) was not available. Thus the volume of distribution divided by fraction of drug absorbed was calculated. The averages (Mean ± S.D.) of this parameter were 1.06 ± 1.69, 0.83 ± 0.26 and 0.74 ± 0.55 liter for the suppository with Eudragit S-100, HP55 and Suppocire ®AM, respectively as shown in Table 34. Statistical comparison (Table 35) reported that all three formulations were not statistically significant differences ($p > 0.05$).

Total plasma clearance (CL/F)

Similar to the volume of distribution, thus the total plasma clearance divided by fraction of drug absorbed was calculated. The average (Mean ± S.D.) total plasma clearance of the formulations with Eudragit S-100, HP55 and Suppocire ®AM

Table 24 The time to peak plasma ketoprofen concentrations (t_{max}) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	t_{max} (hr)		
	Eudragit S-100	HP55	Suppocire ®AM
1	2	1	0.5
2	0.5	1	0.5
3	1	0.5	0.5
4	1	0.5	0.5
5	0.5	0.5	0.5
6	1	1	4
7	0.5	3	2
8	0.5	1	1
9	0.5	1	2
Mean	0.83	1.06	1.28
S.D.	0.50	0.77	1.20

Table 25 Analysis of variance for three way crossover design of time to peak plasma ketoprofen concentrations (t_{max}) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	19.17	0.74	-	-	-
Sequence	2	0.89	0.45	0.45	5.14	NS
Subject (Sequence)	6	6	1	1.91	2.64	NS
Period	2	4.06	2.03	3.87	3.68	S
Formulation	2	0.89	0.45	0.85	3.68	NS
Error	14	7.33	0.52	-	-	-

Table 26 Log of area under plasma ketoprofen concentration-time curves (log AUC) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	log AUC (μg.hr/mL)		
	Eudragit S-100	HP55	Suppocire ®AM
1	2.69	2.68	2.82
2	2.86	2.91	3.01
3	2.84	2.71	3.11
4	2.86	2.83	2.82
5	2.74	2.78	2.72
6	2.81	2.68	2.64
7	2.24	2.56	2.95
8	2.76	2.79	2.89
9	2.92	2.92	2.96
Mean	2.75	2.76	2.88
S.D.	0.27	0.16	0.20

Table 27 Analysis of variance for three way crossover design of log AUC of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05) and 90 percent confidence interval for the difference of AUC means.

Source of variation	Degree of freedom	Sum of squares	Mean square	F-ratio	F-table	Significant level
Total	26	0.71	0.03	-	-	-
Sequence	2	0.04	0.02	0.40	5.14	NS
Subject(Sequence)	6	0.32	0.05	2.50	2.64	NS
Period	2	0.03	0.02	1.00	3.68	NS
Formulation	2	0.10	0.05	2.50	3.68	NS
Error	14	0.22	0.02	-	-	-

1. 90% CI of suppository with Eudragit S-100 = 91.67 – 99.30
2. 90% CI of suppository with HP55 = 92.01 – 99.65

Table 28 Elimination rate constant (K_{el}) of ketoprofen of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	K_{el} (hr^{-1})		
	Eudragit S-100	HP55	Suppocire ®AM
1	0.22	0.23	0.14
2	0.28	0.22	0.27
3	0.24	0.25	0.25
4	0.27	0.20	0.25
5	0.26	0.25	0.26
6	0.24	0.22	0.15
7	0.12	0.23	0.12
8	0.25	0.23	0.25
9	0.23	0.13	0.23
Mean	0.24	0.22	0.21
S.D.	0.06	0.05	0.08

Table 29 Analysis of variance for three way crossover design of elimination rate constant (K_{el}) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	0.0600	0.0020	-	-	-
Sequence	2	0.0054	0.0027	0.54	5.14	NS
Subject(Sequence)	6	0.0300	0.0050	2.50	2.64	NS
Period	2	0.0010	0.0005	0.25	3.68	NS
Formulation	2	0.0008	0.0004	0.20	3.68	NS
Error	14	0.0228	0.002	-	-	-

Table 30 Elimination half-lives ($t_{1/2}$) of ketoprofen of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	$t_{1/2}$ (hr)		
	Eudragit S-100	HP55	Suppocire ®AM
1	3.57	2.71	5.25
2	2.84	2.90	2.46
3	2.99	2.98	3.12
4	2.70	3.29	2.39
5	2.40	3.00	2.16
6	2.88	2.92	6.40
7	6.01	3.31	5.04
8	2.58	2.83	2.58
9	3.10	5.88	3.34
Mean	3.23	3.31	3.64
S.D.	1.46	1.31	2.04

Table 31 Analysis of variance for three way crossover design of elimination half –lives ($t_{1/2}$) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	36.94	1.43	-	-	-
Sequence	2	2.86	1.43	0.54	5.14	NS
Subject(Sequence)	6	15.99	2.66	2.37	2.64	NS
Period	2	1.54	0.77	0.68	3.68	NS
Formulation	2	0.84	0.42	0.37	3.68	NS
Error	14	15.72	1.12	-	-	-

Table 32 Mean residence time (MRT) of ketoprofen of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No.	MRT (hr)		
	Eudragit S-100	HP55	Suppocire ®AM
1	5.14	3.90	7.56
2	4.00	4.18	3.55
3	4.32	4.30	4.50
4	3.90	4.75	3.45
5	3.46	4.33	3.12
6	4.16	4.22	9.23
7	8.67	4.78	7.28
8	3.72	4.09	3.73
9	4.48	8.49	4.81
Mean	4.65	4.78	5.25
S.D.	2.11	1.89	2.95

Table 33 Analysis of variance for three way crossover design of mean residence time (MRT) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of Variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	77.02	2.96	-	-	-
Sequence	2	6.02	3.01	0.54	5.14	NS
Subject(Sequence)	6	33.43	5.57	2.39	2.64	NS
Period	2	3.14	1.57	0.67	3.68	NS
Formulation	2	1.79	0.89	0.38	3.68	NS
Error	14	32.63	2.33	-	-	-

Table 34 Volume of distribution (V_d / F) of ketoprofen of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No	V_d / F (L)		
	Eudragit S-100	HP55	Suppocire ®AM
1	0.92	0.90	1.11
2	0.50	0.56	0.36
3	0.59	0.77	0.32
4	0.51	0.72	0.61
5	0.70	0.68	0.74
6	0.65	0.96	1.59
7	4.42	1.19	0.92
8	0.71	0.72	0.51
9	0.53	0.96	0.48
Mean	1.06	0.83	0.74
S.D.	1.69	0.26	0.55

Table 35 Analysis of variance for three way crossover design of volume of distribution (V_d / F) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	15.008	0.5772	-	-	-
Sequence	2	1.1675	0.5838	0.5300	5.14	NS
Subject (Sequence)	6	6.6089	1.1015	2.6003	2.64	NS
Period	2	0.8065	0.4033	0.9521	3.68	NS
Formulation	2	0.4941	0.2471	0.5833	3.68	NS
Error	14	5.9310	0.4236	-	-	-

were 0.20 ± 0.19, 0.18 ± 0.06 and 0.14 ± 0.06 liter/hour, respectively as shown in Table 36. There were no statistically significant differences ($p>0.05$) of these values (Table 37).

The absorption rate constant (K_a) could not calculated because the data of the mean residence time of intravenous administration (MRT_{iv}), which used to calculate the mean absorption time was not available.

The estimated all pharmacokinetic parameters of ketoprofen from nine rabbits after administration of three formulations of ketoprofen prolonged release rectal suppositories were summarized and shown in Table 38.

3.3.1 Comparison of pharmacokinetic parameters.

The pharmacokinetic parameters obtained from this study slightly revealed intersubject variations among all rabbits even if the design was three way crossovers. Factors affected the variations of these pharmacokinetic parameters might be due to sex, age and weight of rabbits. Nevertheless, the results were not statistically significant differences ($p>0.05$) among these corresponding pharmacokinetic parameters derived from the data obtained of the formulas with Eudragit S-100, HP55 and Suppocire® AM.

3.3.2 Evaluation of bioequivalence.

The 90% confidence interval performed using the data in Table 22-23 and 26-27 for the differences of C_{max} and AUC means based on log transformed data of the formulations with Eudragit S-100 and HP55 relative to the reference formulation using Suppocire ®AM were within 80-125%. This could be concluded that the three formulations of 100 mg prolonged release rectal suppositories were bioequivalent in terms of both the rate and the extent of drug absorption into systemic circulation.

Table 36 Total plasma clearance (CL / F) of ketoprofen of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Rabbit No	CL / F (L/hr)		
	Eudragit S-100	HP55	Suppocire ®AM
1	0.20	0.21	0.15
2	0.14	0.12	0.10
3	0.14	0.19	0.08
4	0.14	0.15	0.15
5	0.18	0.17	0.19
6	0.16	0.21	0.23
7	0.57	0.27	0.11
8	0.17	0.16	0.13
9	0.12	0.12	0.11
Mean	0.20	0.18	0.14
S.D.	0.19	0.06	0.06

Table 37 Analysis of variance for three way crossover design of total plasma clearance (CL / F) of nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories (α=0.05).

Source of variation	Degree of freedom	Sum of squares	Mean Square	F-ratio	F-table	Significant level
Total	26	0.21	0.008	-	-	-
Sequence	2	0.01	0.005	0.35	5.14	NS
Subject (Sequence)	6	0.09	0.02	2.48	2.64	NS
Period	2	0.008	0.004	0.66	3.68	NS
Formulation	2	0.02	0.01	1.56	3.68	NS
Error	14	0.08	0.006	-	-	-

Table 38 Estimated pharmacokinetic parameters of ketoprofen (Mean ± S.D.) from nine rabbits after administration of three formulations of 100 mg prolonged release ketoprofen rectal suppositories.

Pharmacokinetic parameters	Formulations			Statistical Test
	Eudragit S-100	HP55	Suppocire® AM	
log C_{max} (μg/mL)	2.12 ± 0.33	2.11 ± 0.21	2.19 ± 0.35	NS
log AUC (μg.hr/mL)	2.75 ± 0.27	2.76 ± 0.16	2.88 ± 0.20	NS
t_{max} (hr)	0.83 ± 0.50	1.06 ± 0.77	1.28 ± 1.20	NS
K_{el} (hr^{-1})	0.24 ± 0.06	0.22 ± 0.05	0.21 ± 0.08	NS
$t_{1/2}$ (hr)	3.23 ± 1.46	3.31 ± 1.31	3.64 ± 2.04	NS
MRT (hr)	4.65 ± 2.11	4.78 ± 1.89	5.25 ± 2.95	NS
V_d/F (liter)	1.06 ± 1.69	0.83 ± 0.26	0.74 ± 0.55	NS
CL/F (liter/hr)	0.20 ± 0.19	0.18 ± 0.06	0.14 ± 0.06	NS

NS = Not significant difference ($p>0.05$).

3.4 Comparison of the efficacies of Eudragit S-100 and HP55 on ketoprofen rectal suppositories as prolonged release carriers.

Both the *in vitro* and *in vivo* data obtained in this study revealed that the effects or performance of Eudragit S-100 and HP55 on ketoprofen rectal suppositories as prolonged release carriers were equally similar. Both formulations could be used interchangeably. However, owing to the ratio of drug to carrier, the ease of preparation, Eudragit S-100 appeared to be superior to HP55 as prolonged release carrier.