CHAPTER III

RESULTS

In Vitro Evaluation of NFP Transdermal Patch

All prepared NFP transdermal patches featured yellowish gel according to the color of drug. Each formulation was filled into an aluminium mold with a fixed circular diameter at 3.44 cm, a fixed depth at 1.5 mm, and 9.2941 cm^2 surface area before evaluating the physical properties, as shown in Figure 11.

Physical properties

The physical properties data such as difficulty in preparing, clarity,air bubles, and residue on applications of twenty formulations (F1-F20) are summarized in Table 2.

Difficulty in preparing

The degree of difficulty were measured by the times and forces used in preparing each formulation. They were different due to various types and concentrations of surfactants used. In the formulation containing Cremophor A25 (F9 and F10), stearyl alcohol (F17 and F18), and cetyl alcohol (F19 and F20), they took longer times to disperse surfactant completely into Pluronic F-127 gel than the others.

Figure 11 **Nifedipine transdermal patch prepared in this study**

Table 2 Physical properties of NFP transdermal patch using various types and concentration of surfactants

Physical Properties	Preparation Number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Difficulty in preparing[1]	-	-	-	-	-	-	-	-	+	+2	-	-	-	-	-	-	+2	+2	+2	+2
Clarity[2]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Air bubble[1]	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+
Residue on application[1]	-	-	+	+	-	+	-	-	+	+	+2	+2	+2	+3	+3	+3	+2	+3	+2	+3

1. The number of the symbols of (+) and (-) showed a degree of intensity and no appearance, respectively

2. Clarity : (+) = transparent, (-) = translucent

It was found that increasing the concentration of Cremophor A25 from 1% (F9) to 2% (F10) increased the time in preparing. However, the concentration of surfactant did not affect the times used in formulations containing stearyl alcohol (F17 and F18) and cetyl alcohol (F19 and F20). These four formulations took approximate equal times in preparing.

Clarity

All ingredients in the formulation were dissolved in the gel base. The appearance of each product was transparent without any insoluble materials in preparations.

Air bubbles

Small air bubbles appeared in the gels during preparing process and were prominent in formulations containing stearyl alcohol (F17 and F18) and cetyl alcohol (F19 and F20). In the other formulations, air bubbles disappeared after stored the mixture in refrigerator. It was found that the amount of small air bubbles in the formulations containing 1% stearyl and cetyl alcohol (F17 and F19) were less than the amount of them in formulations containing 2% stearyl and cetyl alcohol (F18 and F20).

Residue

No residue could be observed on the applied area from formulations containing no surfactant (F1), 0.5% sodium lauryl sulfate (F2), 1% cetyl trimethylammonium bromide (F5), and 1, 2 % cetylpyridinium chloride (F7 and F8).

For formulations containing 1 and 2 % sodium lauryl sulfate (F3 and F4), 2% cetyltrimethylammonium bromide (F6), 1 and 2% Cremophor A25 (F9 and F10), some residue (+1) could be detected on the applied area. More residue (+2) could be observed on the applied area from formulation containing 1 and 2% Cremophor RH40 (F11 and F12), 1% stearic acid (F13), 1% stearyl alcohol (F17), and 1% cetyl alcohol (F19). Formulations containing 2% stearic acid (F14), 1 and 2% oleic acid (F15 and F16), 2% stearyl alcohol (F18), and 2% cetyl alcohol(F20) exhibited the highest grease-like residue (+3) when applied on the surface of skin

From acceptable physical characteristics according to the comfortably in preparing, clarity of gel, disappearance of air bubbles prominent, and good consistency, ten formulations selected for further *in-vitro* study were F1 to F10. These were formulations contained no surfactant (F1), 0.5,1, and 2% sodium lauryl sulfate (F2, F3, and F4), 1 and 2% cetyltrimethylammonium bromide (F5 and F6), 1 and 2% cetylpyridinium chloride (F7 and F8), 1 and 2% Cremophor A25 (F9 and F10).

Chromatographic condition

The chromatographic condition used for this experiment could provide good resolution as shown in Figure 12. Internal standard (IS), 4-dimethyl aminobenzaldehyde, and NFP were eluted at the retention times of 5.63-5.71 and 6.57-6.69 minutes, respectively. The run time per sample was within 10 minutes. Either standard or sample solution gave similar retention times for both drug and IS as shown in Figure 13.

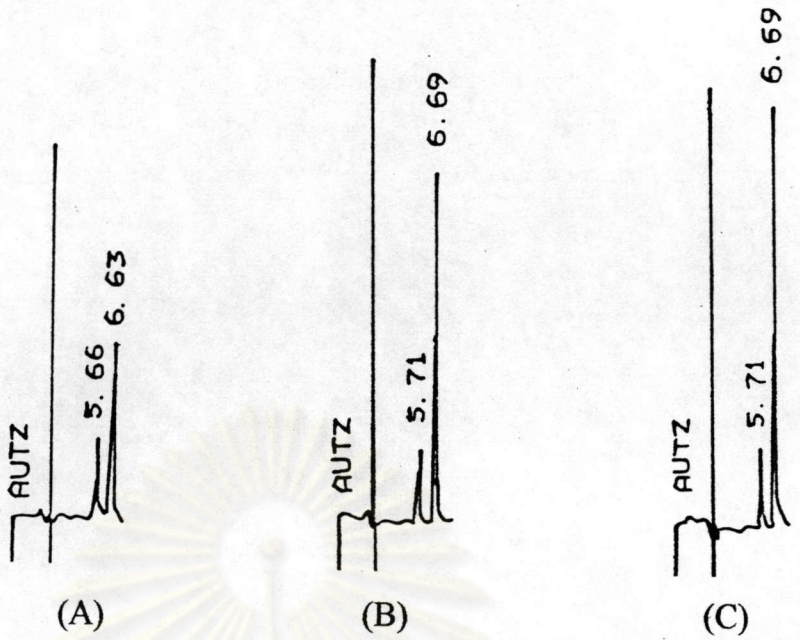


Figure12 Chromatogram of NFP from *in vitro* permeation study and 4-dimethylaminobenzaldehyde(IS) 0.12 μg/ml in methanol at 238 nm

(A) NFP 240 μg/ml (B) NFP 480 μg/ml (C) NFP 600 μg/ml

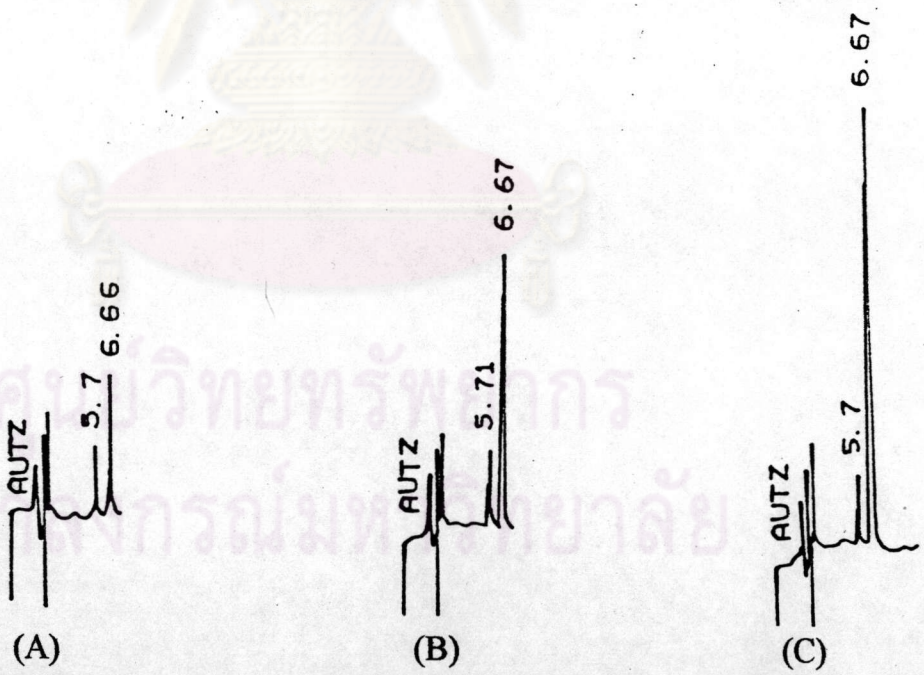


Figure 13 Chromatogram of NFP from *in vitro* permeation study and 4-dimethylaminobenzaldehyde(IS) 0.12 μg/ml at 238 nm

(A) At 7 hr (B) At 10 hr (C) At 13 hr

Calibration curve

The calibration curve of NFP was constructed by plotting between the peak area ratio of NFP and IS versus NFP concentration. Calibration curve in Figure 14 represents linear regression line between PAR and NFP concentration according to the equation of

$$Y = 11.64X - 0.12 \qquad \text{(3)}$$

where Y was the PAR of NFP versus IS and X was the concentration of NFP. Correlation coefficient of the plot (r) was 0.9981. Calibration curve was constructed for each day of sample analysis.

***In vitro* evaluation of NFP TDDs formulations**

NFP permeation data through pig's skin from *in-vitro* permeation study are shown in Table 3 and Appendix I. NFP permeation-time profiles are shown from Figures 15 to Figure 21. The permeation data were presented as average cumulative permeation of NFP per unit surface area of pig's skin (Qs).

Figure 15 shows that all formulations (F1-F10) could provide sustained permeation pattern of NFP over 24 hours. During the initial stage there was a characteristic lag phase followed by diffusion stage. The maximum permeation amount was observed from formulation containing 2% cetyltrimethylammonium bromide (F6) to be 340.32 $\mu g/cm^2$ at 24 hr and 368.25 $\mu g/cm^2$ at 28 hr whereas the minimum permeation amount of 92.74 $\mu g/cm^2$ was observed from formulation containing 1% cetyltrimethyl ammonium bromide (F5). The cumulative permeation amount of NFP at 24

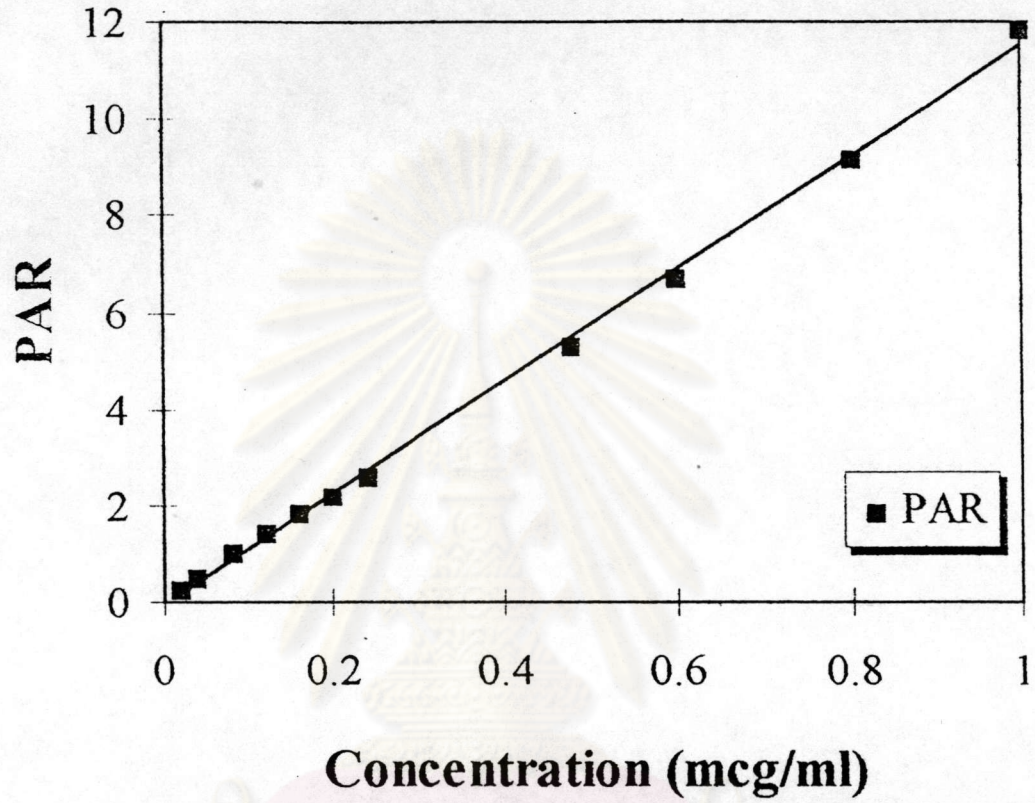


Figure 14 Calibration curve of NFP-IS peak area ratio (PAR) as a function of NFP concentration range of 0.02-1.00 μg/ml in mobile phase (Y = 11.64 X- 0.12 ; r^2 = 0.9981)

Table3 The average cumulative amount of NFP per surface area of pig's skin permeation from NFP transdermal patch using various types and concentrations of surfactants.

Time (hr)	Average cumulative permeation amount/surface area ($\mu g/cm^2$)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	3.02	2.64	1.05	0.98	1.77	4.64	2.55	0.73	0.43	1.15
2	6.36	3.98	2.30	1.13	5.59	6.31	3.93	1.89	1.12	0.65
4	9.59	9.48	4.63	1.28	4.65	11.36	5.87	5.64	3.15	1.27
7	10.53	20.91	6.24	1.49	7.45	18.97	9.35	12.26	9.23	2.59
10	15.89	35.07	10.63	5.79	9.12	43.12	15.90	20.46	18.65	6.69
13	35.12	88.73	22.44	37.83	15.59	76.38	38.72	32.89	29.39	15.01
16	67.39	147.39	53.70	97.40	29.93	132.76	59.91	54.01	36.06	30.05
20	93.07	171.51	97.59	148.33	58.61	220.95	79.46	89.04	99.63	88.47
24	142.44	202.08	181.34	245.20	92.74	340.32	147.34	119.78	123.77	102.29
28	-	233.38	-	-	-	368.25	-	161.78	208.29	151.55

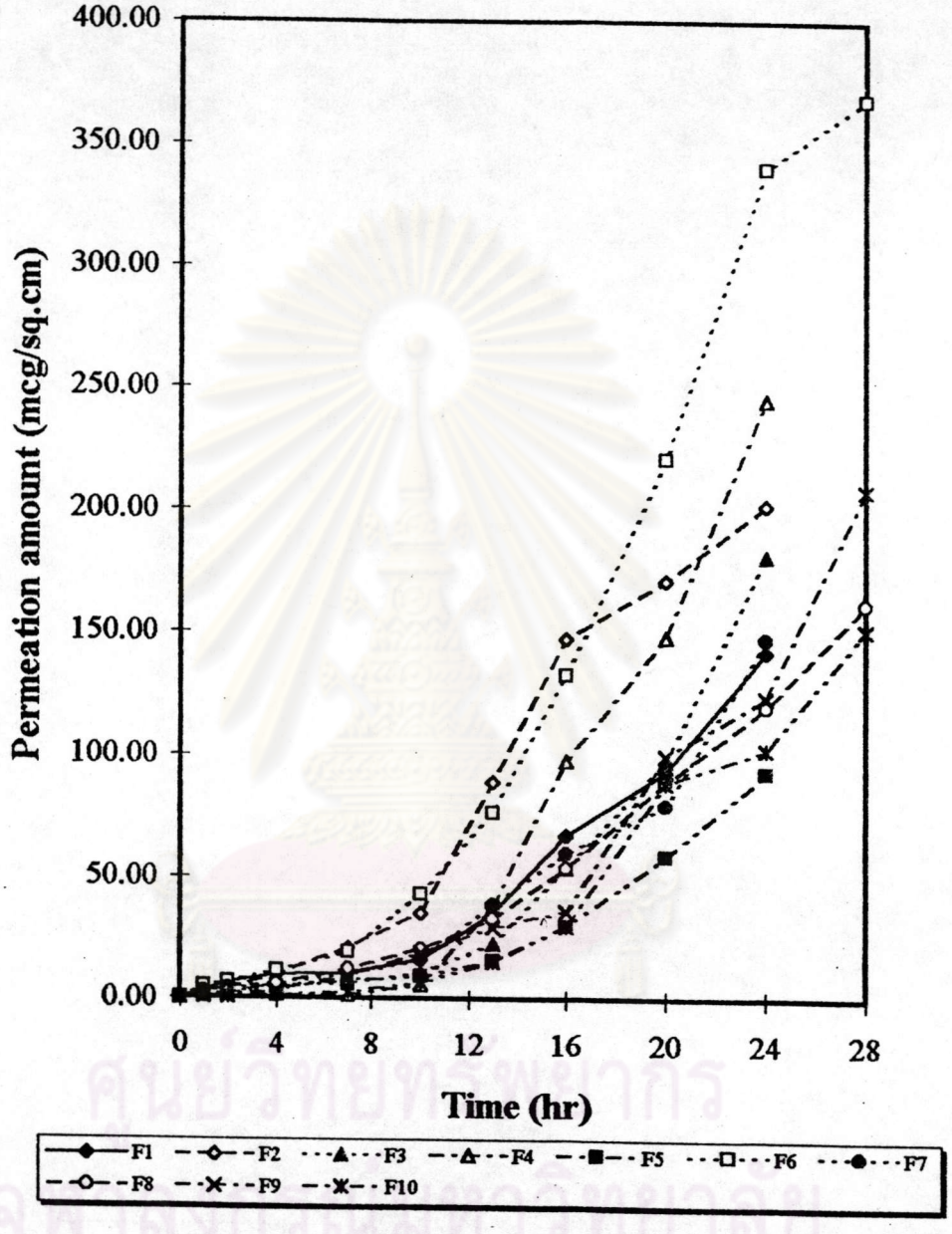


Figure 15 Drug permeation-time profiles of NFP transdermal patch from F1-F10

. hr could be ranked as F6 >> F4 > F2 > F3 > F7 ≥ F1 > F9 ≥ F8 >F10> F5.

The surfactants used as enhancer on skin permeation of NFP were divided into three groups by types of surfactants, such as anionic-, cationic-, and nonionic- surfactant. The results were relatively compared with control formulation (F1) which contained no surfactant.

The effects of anionic surfactant on skin permeation amount of NFP were investigated by comparing the relationship of drug permeation-time profiles from formulations containing no surfactant (F1), with those of 0.5% sodium lauryl sulfate (SLS) (F2), 1.0% SLS (F3), and 2.0 % SLS (F4). The results are shown in Figure 16. During the permeation study, no drug was permeated from each formulation in the first two hours, then the permeation of NFP was slowly increased. After 10 hours, permeation amount was rapidly increased to the maximum amount after 24 hours.

At time interval 4-20 hours, the permeation amount from NFP transdermal patch with 0.5% SLS (F2) rapidly increased higher than from F1, F3, and F4 that contained no surfactant, 1% SLS, and 2% SLS, respectively. However at 24 hours, the maximum amount among this group was observed from the formulation containing 2% SLS (F4).

The fluxes across pig's skin from NFP transdermal patch containing cationic surfactant and no surfactant are shown in Figure 17. It could be seen that the highest amount permeated from formulation containing 2% cetyl trimethylammonium bromide (F6). At 24 hr, it increased cumulative permeation amount about 2 times comparing to control formula (F1).

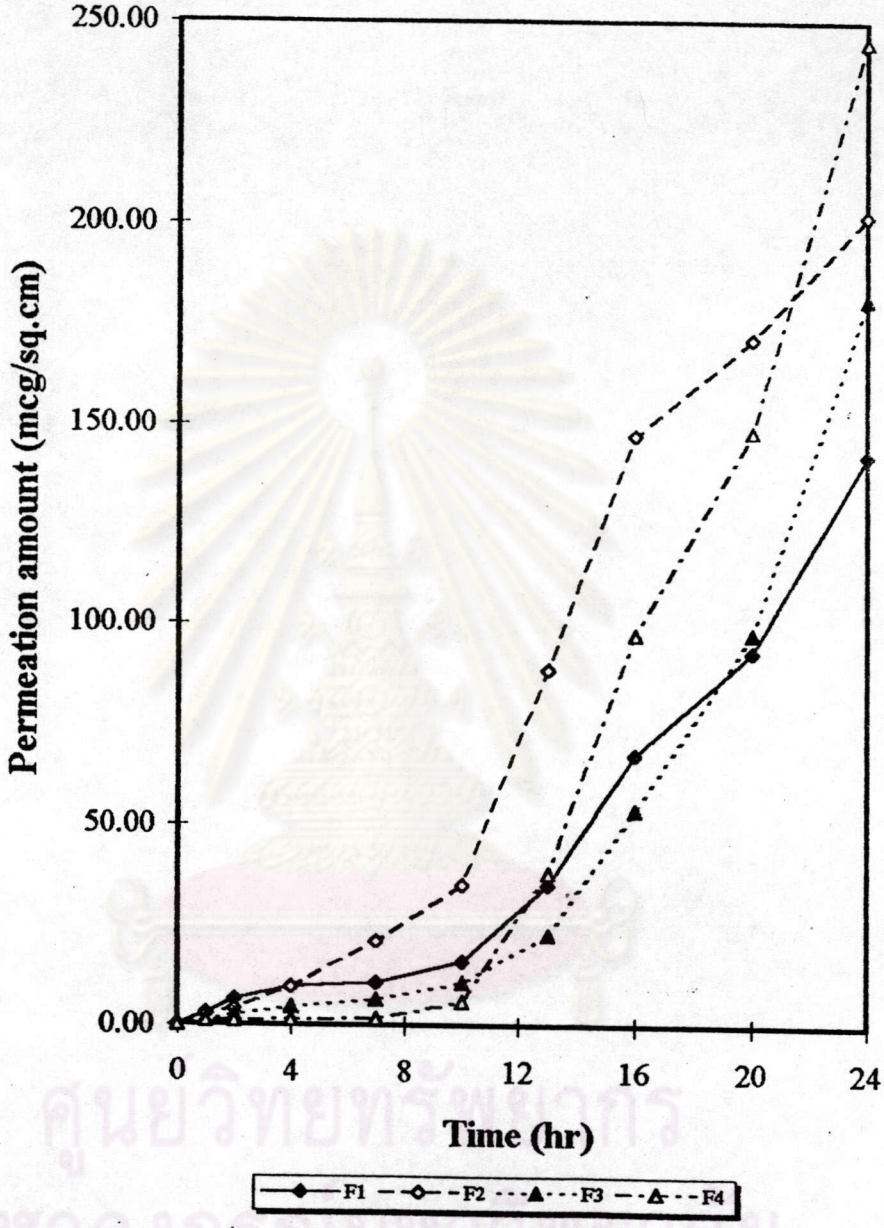


Figure 16 Drug permeation-time profiles of NFP transdermal patch from F1 vs F2, F3, and F4 (contained anionic surfactant)

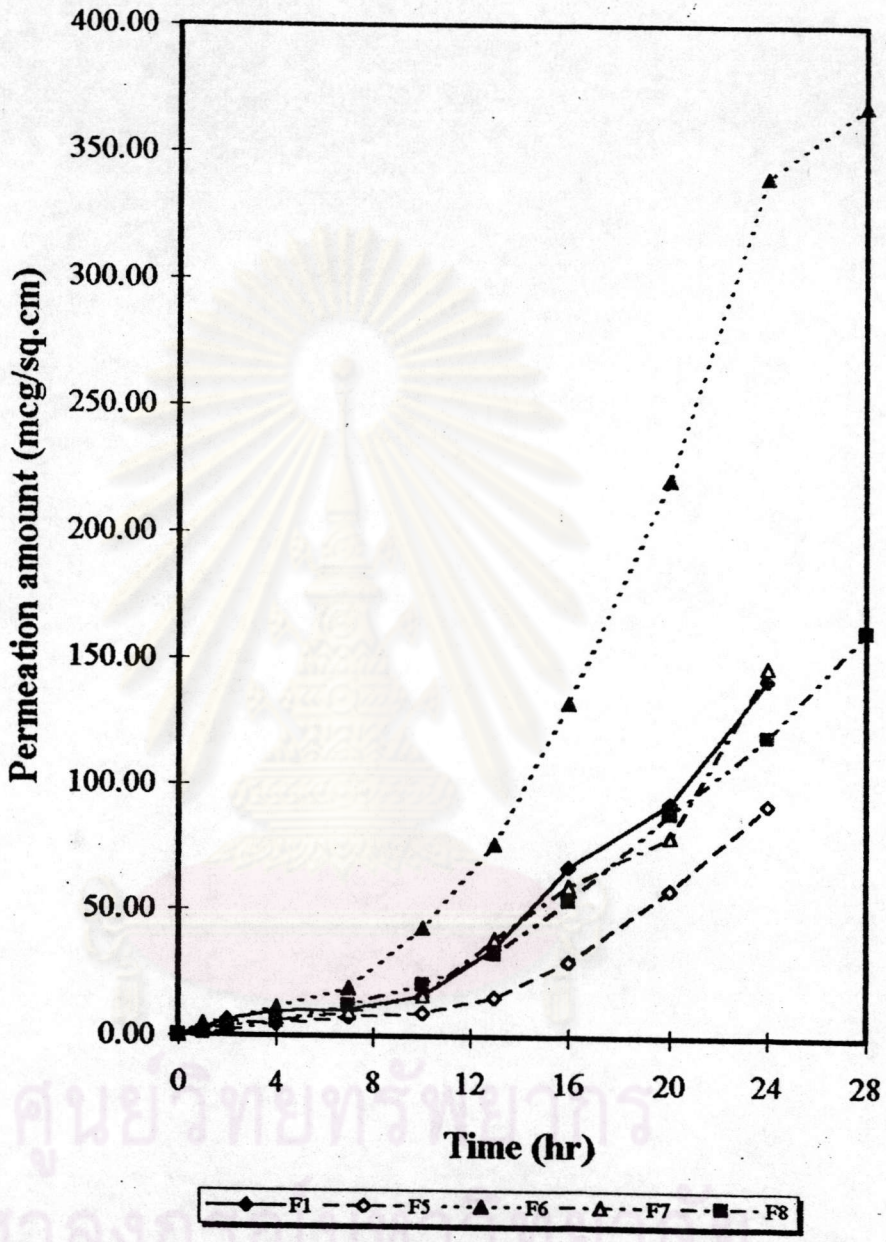


Figure 17 Drug permeation-time profiles of NFP transdermal patch from F1 vs F5, F6, F7, and F8 (contained cationic surfactant)

Comparison of permeation amount between formulation containing 1 and 2 % cetyltrimethylammonium bromide (F5 and F6) are shown in Figure 18. The results revealed that F5 exhibited lower amount than F6 and F1 about 3 and 1 fold. F5 was the lowest permeated formulation in this group.

The effects of cetylpyridinium chloride on the skin permeation profile of NFP were observed from F7 and F8 (contained 1 and 2 % cetylpyridinium chloride), as depicted in Figure 19. The results of permeation profiles were similar to control formulation.

The effects of nonionic surfactant on skin permeation profiles are shown in Figure 20. In this group consisted of formulation containing 1 and 2 % Cremophor A25 (F9 and F10). It was found that skin permeation rate of NFP was decreased when increasing concentration of Cremophor A25. During the time of experiment, F1 had more permeation amount than F9 and F10. Except for the time at 10 and 20 hr, the cumulative permeation amount from F9 was higher than F1.

Comparison of the highest cumulative permeation amount from each group of surfactant are shown in Figure 21, which obtained from the formulation containing no surfactant (F1), 0.5% SLS (F2), 2% cetyltrimethylammonium bromide (F6), and 1% Cremophor A25 (F9). It was noticed that the formulation containing cationic surfactant (F6) exhibited faster and higher NFP-permeation rate from devices when compared to other formulations.

The permeation pattern of NFP transdermal patches were determined by linear correlation coefficient. The relationship between NFP permeation versus time and versus square root time were shown in Table 4, the

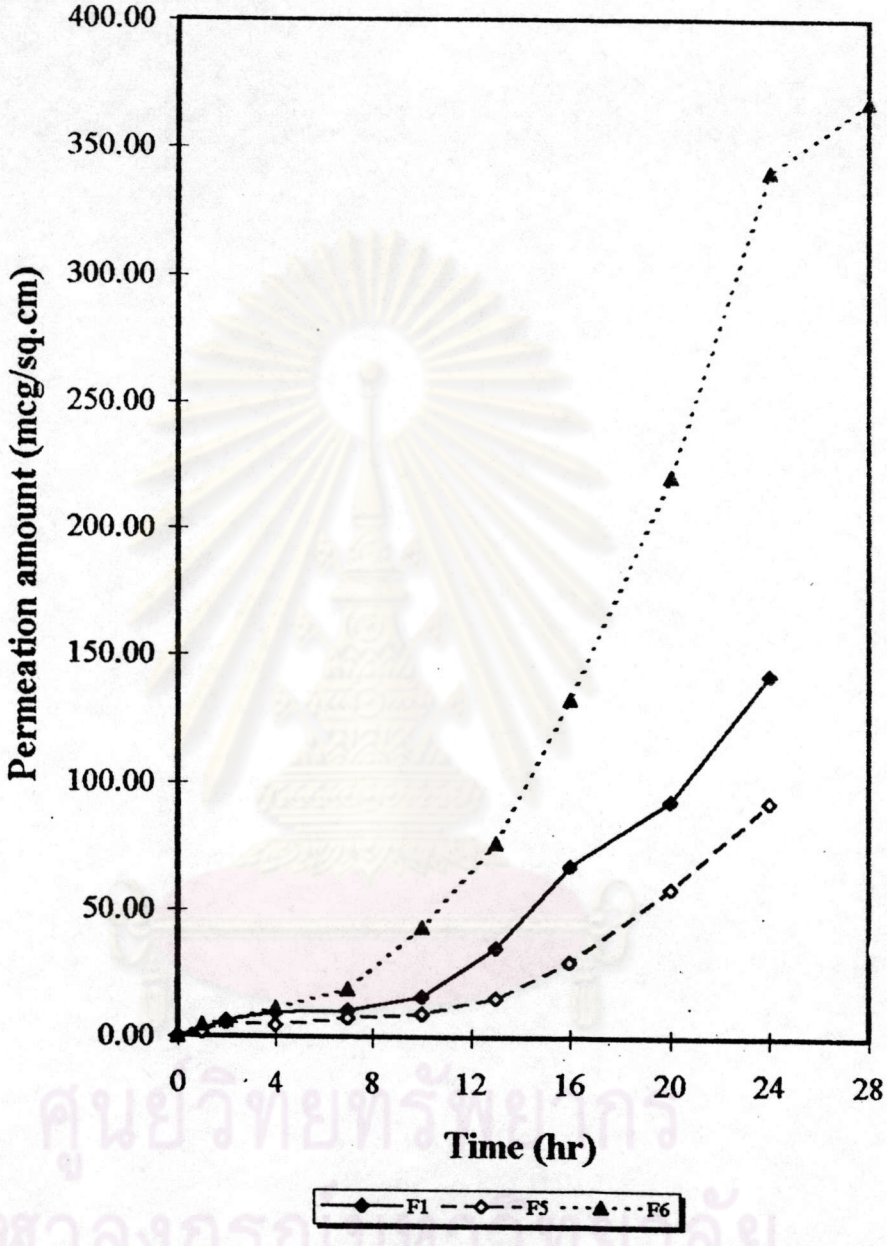


Figure 18 Drug permeation-time profiles of NFP transdermal patch from F1 vs F5 and F6 (contained cetyltrimethylammonium bromide)

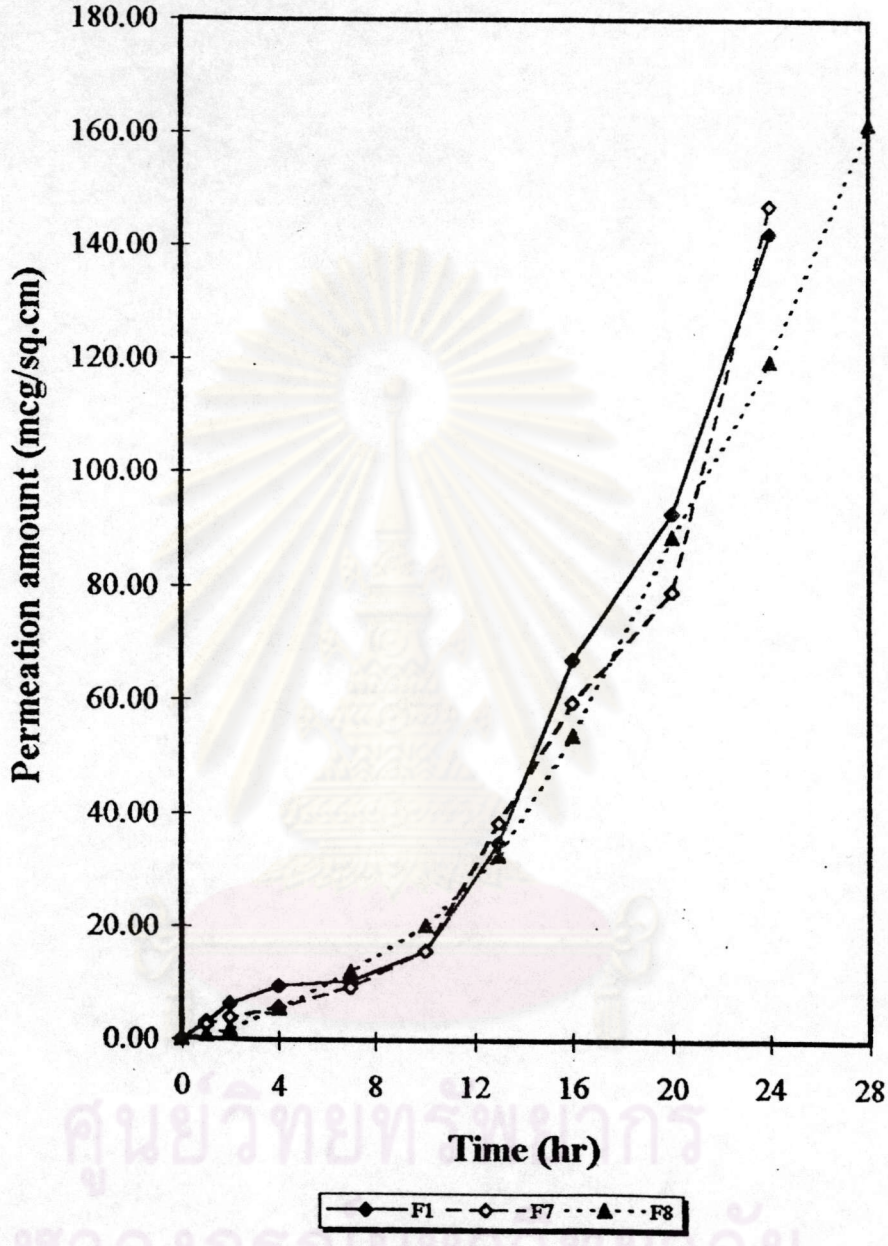


Figure 19 Drug permeation-time profiles of NFP transdermal patch from F1 vs F7 and F8 (contained cetylpyridinium chloride)

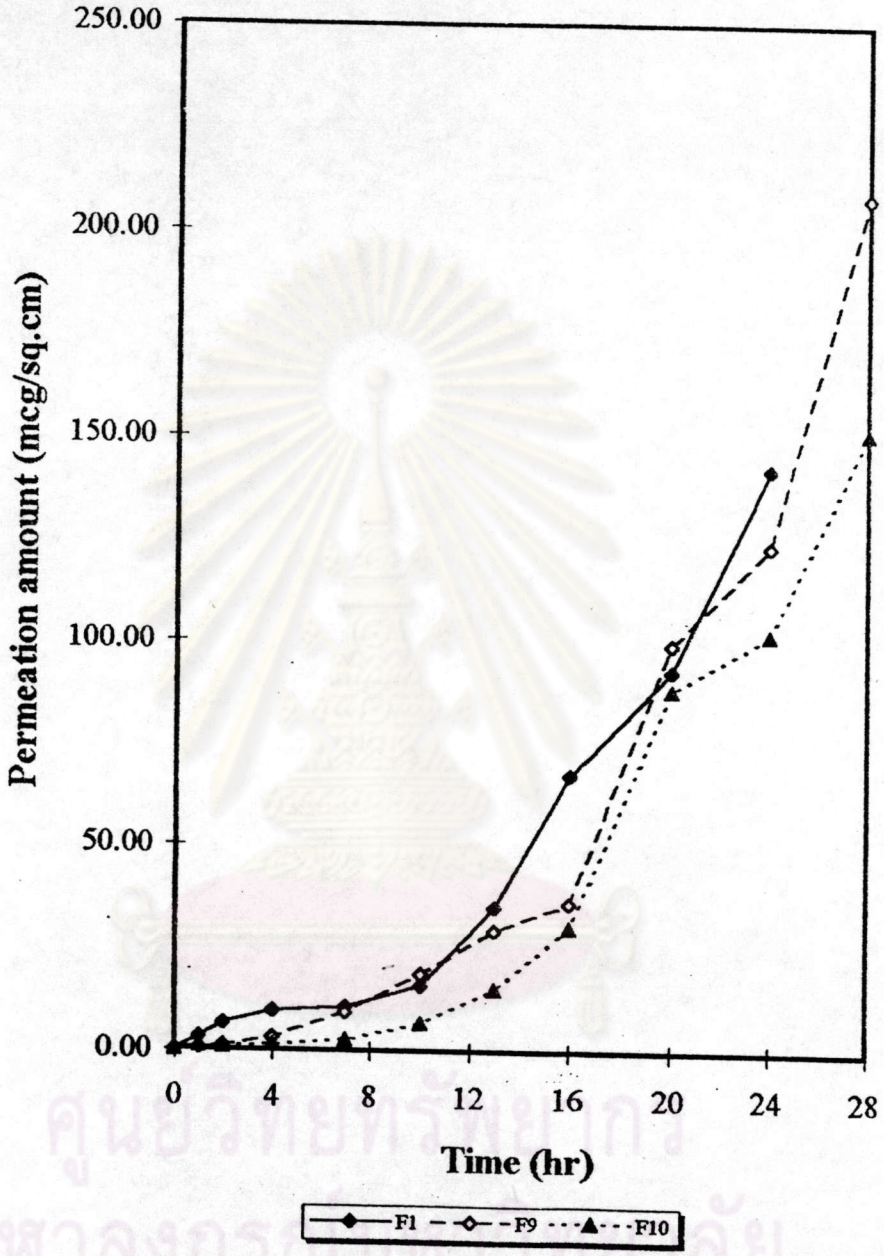


Figure 20 Drug permeation-time profiles of NFP transdermal patch from F1 vs F9, and F10 (contained nonionic surfactant)

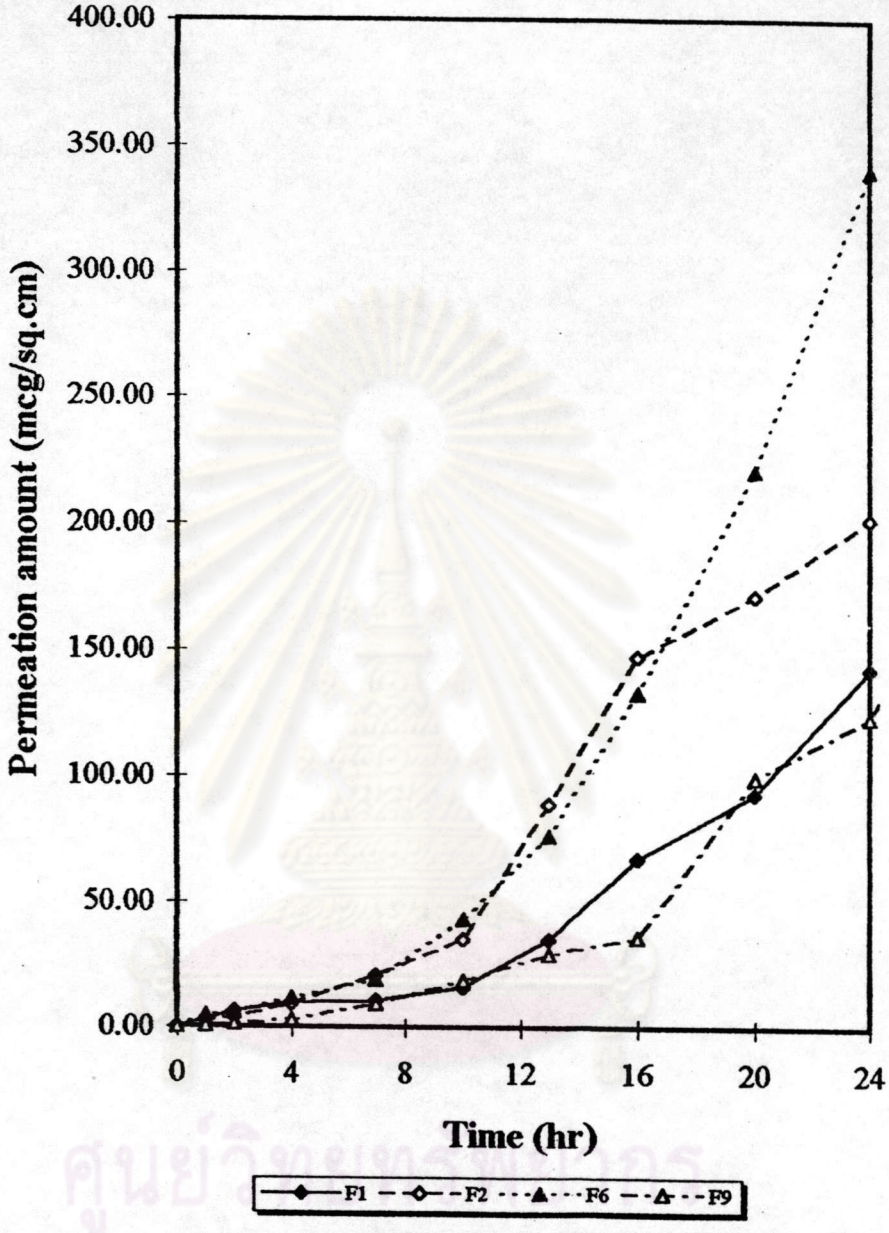


Figure 21 Drug permeation-time profiles of NFP transdermal patch from F1 vs F2, F6, and F9

permeation profile better fitted with a zero order kinetic than that followed Higuchi's model. This trend of kinetic pattern could be better described by the higher correlation coefficient in the relationship between the NFP permeation amount against time.

Comparing the NFP permeation profiles of all formulations suggested that each permeation profile could be divided into two phases, the first phase was 0 to 10 hr and the second phase was 10 to 24/28 hr. The NFP permeation rate and correlation coefficient of all formulations in the first phase were 0.4596-3.9066 $\mu g/cm^2 hr$ and 0.7571-0.9826, respectively. In the second phase, the permeation rate and correlation coefficient were 6.0980-19.7972 $\mu g/cm^2 hr$ and 0.9273-0.9881, respectively, as shown in Table 5. It was found that the permeation rate in the first phase was slower than the second phase.

The effects of types and concentrations of surfactants on skin permeation profile of NFP transdermal patch by *in vitro* permeation experiment could be concluded that the formulation containing 2% cetyltrimethylammonium bromide (F6) had the highest permeation amount and rate in this experiment and was selected for *in vivo* study.

Table 4 Correlation coefficient of the relationship between cumulative NFP permeation versus time (A), cumulative NFP permeation versus square root time (B) and kinetic pattern.

Formulation	A			B		
	correlation coefficient	Y-intercept	X-coefficient	correlation coefficient	Y-intercept	X-coefficient
F1	0.8844	-13.7307	5.3681	0.7036	-29.7928	25.0498
F2	0.9429	-19.9393	9.0856	0.7964	-50.6252	43.6842
F3	0.7826	-22.7205	6.2590	0.5834	-38.9042	28.2718
F4	0.8189	-34.5102	9.1189	0.6137	-58.3853	41.2988
F5	0.8187	-9.2836	3.2813	0.6301	-18.4154	15.0596
F6	0.9139	-43.7624	13.6356	0.7397	-94.3800	69.5971
F7	0.8627	-14.8715	5.2757	0.6794	-30.3160	24.4933
F8	0.9244	-17.0625	5.4893	0.7513	-37.6111	28.0760
F9	0.8427	-24.8138	6.4214	0.6533	-46.5936	32.0787
F10	0.8450	-20.7380	5.0220	0.6480	-37.4160	24.9700

Table 5 Permeation rate and correlation coefficient between cumulative NFP permeation versus time in the first and the second phase of NFP permeation profiles.

Formulation	The first phase, 0-10 hr		The second phase, 10-24/28 hr	
	Permeation rate (mcg/cm^2hr)	Correlation coefficient	Permeation rate (mcg/cm^2hr)	Correlation coefficient
F1	1.4209	0.9251	8.9185	0.9870
F2	3.4766	0.9787	11.6812	0.9373
F3	1.0101	0.9826	12.0987	0.9412
F4	0.4596	0.7571	16.9315	0.9830
F5	0.8186	0.8471	6.0980	0.9601
F6	3.9066	0.9239	19.7972	0.9778
F7	1.4586	0.9779	8.7679	0.9412
F8	2.0753	0.9808	7.9457	0.9881
F9	1.8386	0.9353	10.3612	0.9273
F10	0.5832	0.8562	8.2958	0.9626

In-Vivo Evaluation of NFP Formulations

Method of analysis

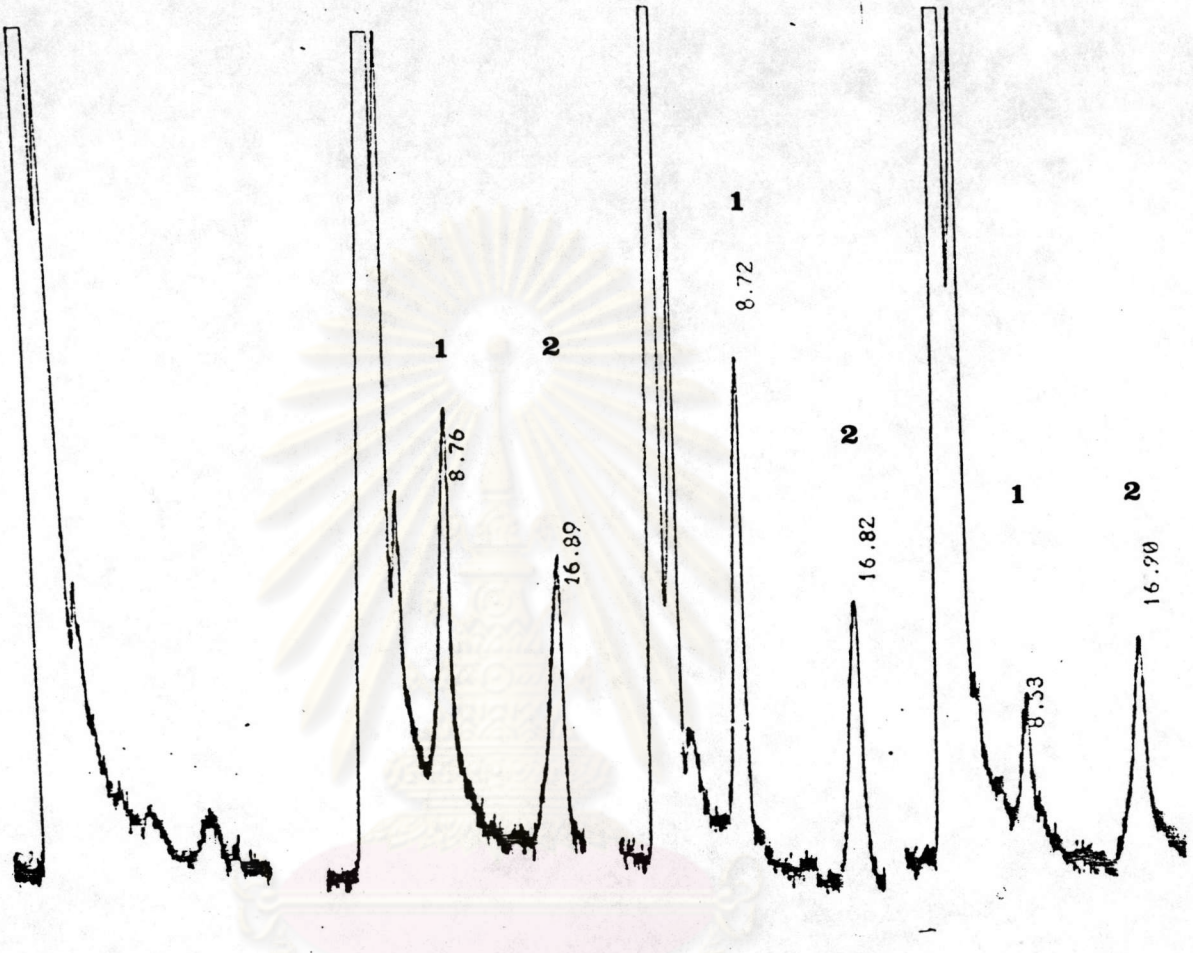
The HPLC technique for determining NFP in plasma sample was modified from Thongnopnua and Wiwattanawongsa's (1992). By changing ionic strength of mobile phase used, the chromatogram of NFP can be better resolved, as demonstated in Figure 22.

NFP and IS were eluted at approximately 8.53-8.76 and 16.82-16.90 minutes, respectively. Either spiked or plasma samples obtaining following oral and transdermal administrations gave similar retention times for both drug and IS. No interfering peaks were observed in these regions. These supported the specificity of the method used for this study.

The relationship between the peak height ratio of NFP/IS (PHR) and NFP concentration (Conc.) was determined in the term of power function in which the represented equation for this relationship could be shown as

$$PHR = 0.04\ (Conc.)^{0.98} \qquad \text{____ (4)}$$

Linear calibration curve could be obtained from log-log plot of PHR and NFP concentration, as shown in Figure 23, with correlation coefficient (r) of 0.9996.



(A) (B) (C) (D)

Figure 22 Chromatograms from rabbit's samples

(A) Blank plasma

(B) Plasma spiked with standard solution of NFP 240 ng/ml

(C) Plasma sample taken after 1 hr of oral administration

(D) Plasma sample taken after 1 hr of transdermal patch administration

Peak identification : (1) nifedipine; (2) IS

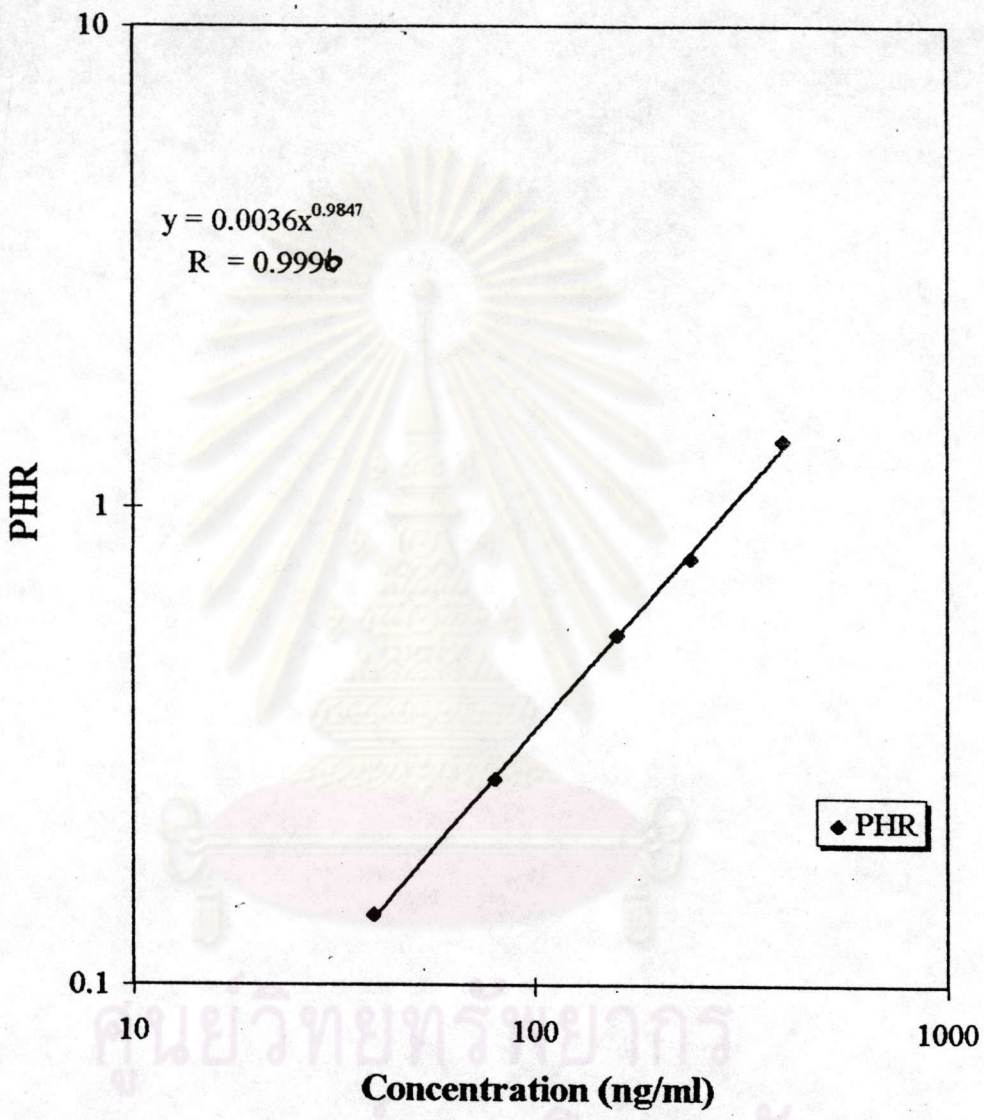


Figure 23 Calibration curve of NFP-IS peak height ratio (PHR) as a function of NFP concentration in rabbit plasma

***In vivo* evaluation of NFP in rabbit plasma**

The normalized NFP plasma concentration-time profiles from individual rabbit for both oral and transdermal patch administration are shown from Figure 24 to Figure 30 . After oral NFP administration (Adalat ®10 mg) to rabbits, t_{max} were within the range of 0.5-12 hr. C_{max} determined from the profiles were within 451.5-1108.1 ng/ml. The detail data are shown in Table 6.

The time to reach maximum NFP concentration for every rabbit using transdermal patch was at the same time point at 8 hr. with the maximum concentration range 47.2-218.3 ng/ml, as shown in Table 7.

Comparison of the normalized maximum NFP concentration (C_{max}) between oral and transdermal patch using unpaired t-test. It was found that the normalized C_{max} from oral administration was statistically significant higher than normalized C_{max} from transdermal patch administration ($p < 0.001$).

The area under NFP plasma concentration-time curve of all five rabbits administering oral and transdermal patch were determined from zero to 24 hrs after drug administration ($AUC\,|_{0}^{24}$) as shown in Table 8. Since the dose for oral and transdermal patch for every rabbit were 10 and approximately 30 mg, respectively, the normalized area were also calculated. It was found that the normalized $AUC\,|_{0}^{24}$ from oral administration was statistically significant higher than normalized $AUC\,|_{0}^{24}$ from transdermal patch administration ($0.001 < p < 0.005$).

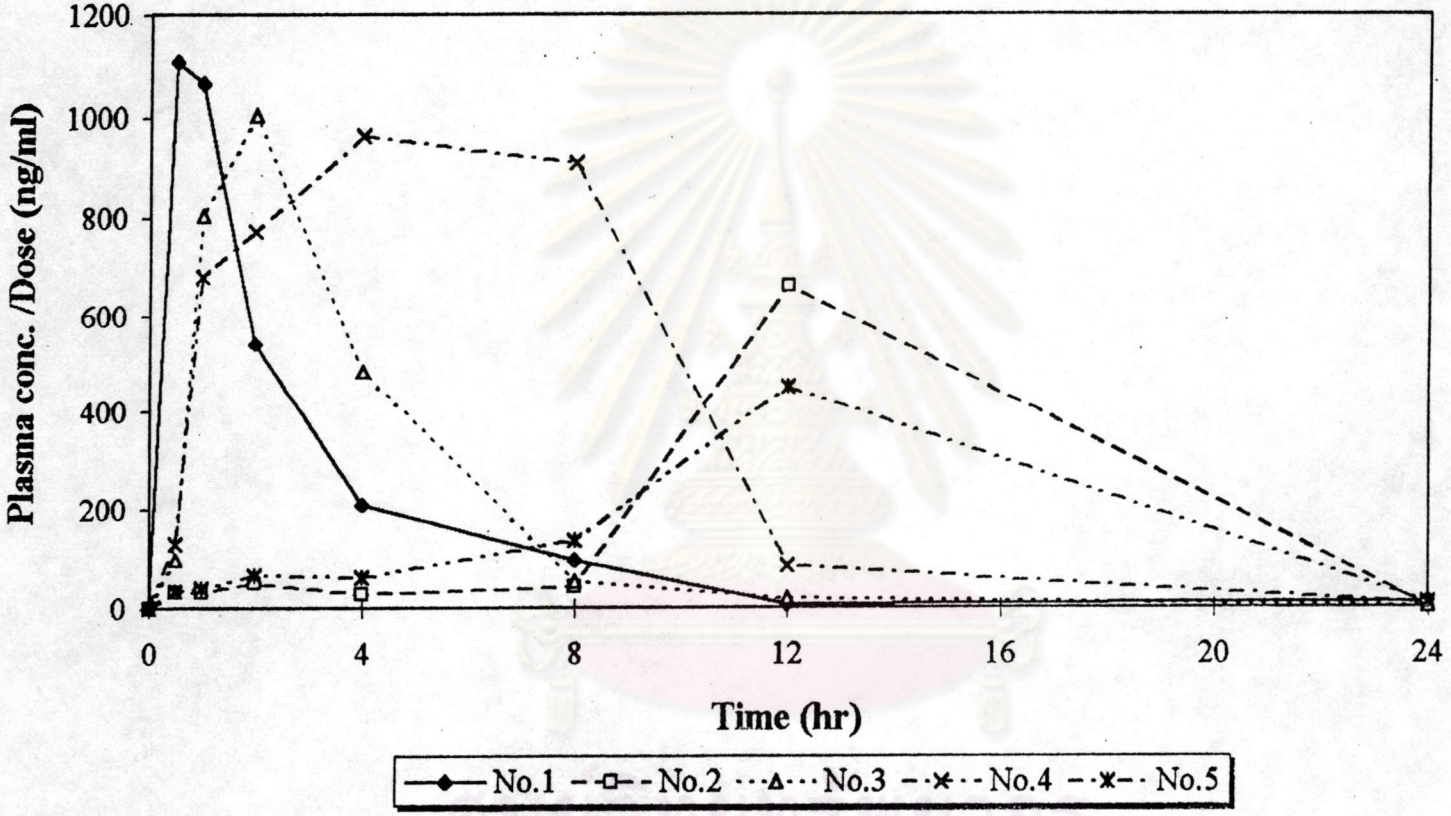


Figure 24 Normalized plasma concentration-time profiles of NFP from adalat® 10 mg

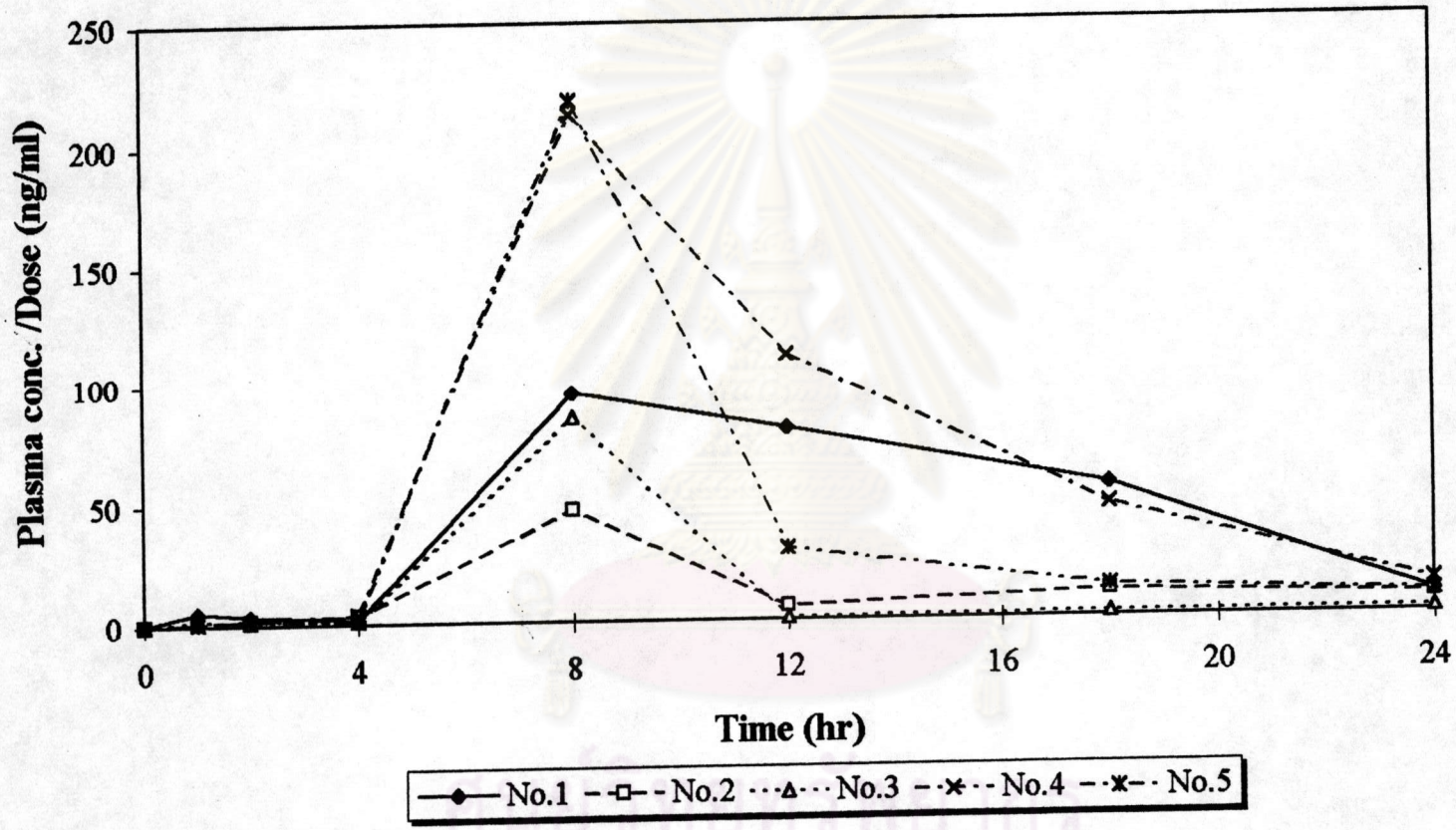


Figure 25 Normalized plasma concentration-time profile of NFP from transdermal patch

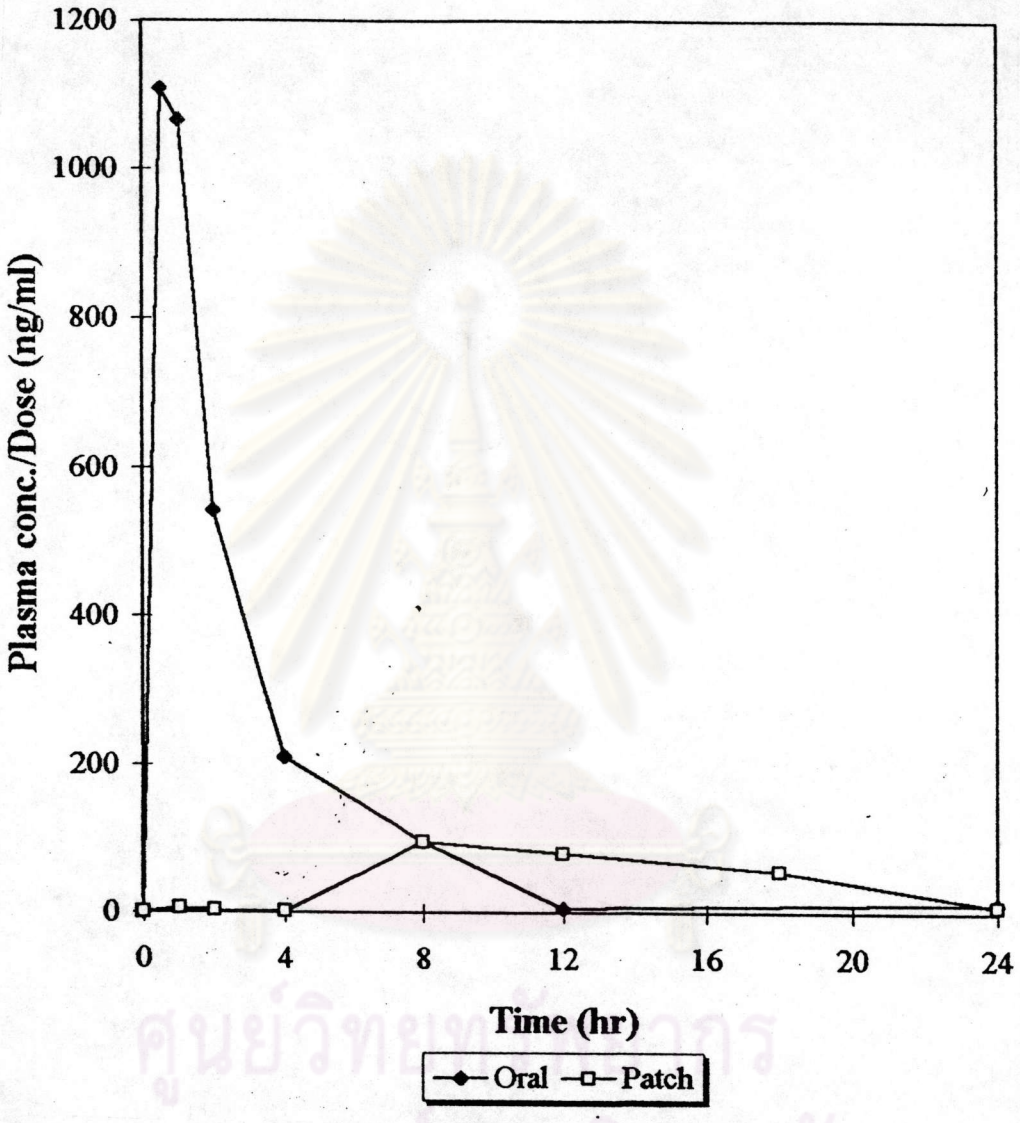


Figure 26 Normalized plasma concentration-time profiles of NFP from rabbit No.1

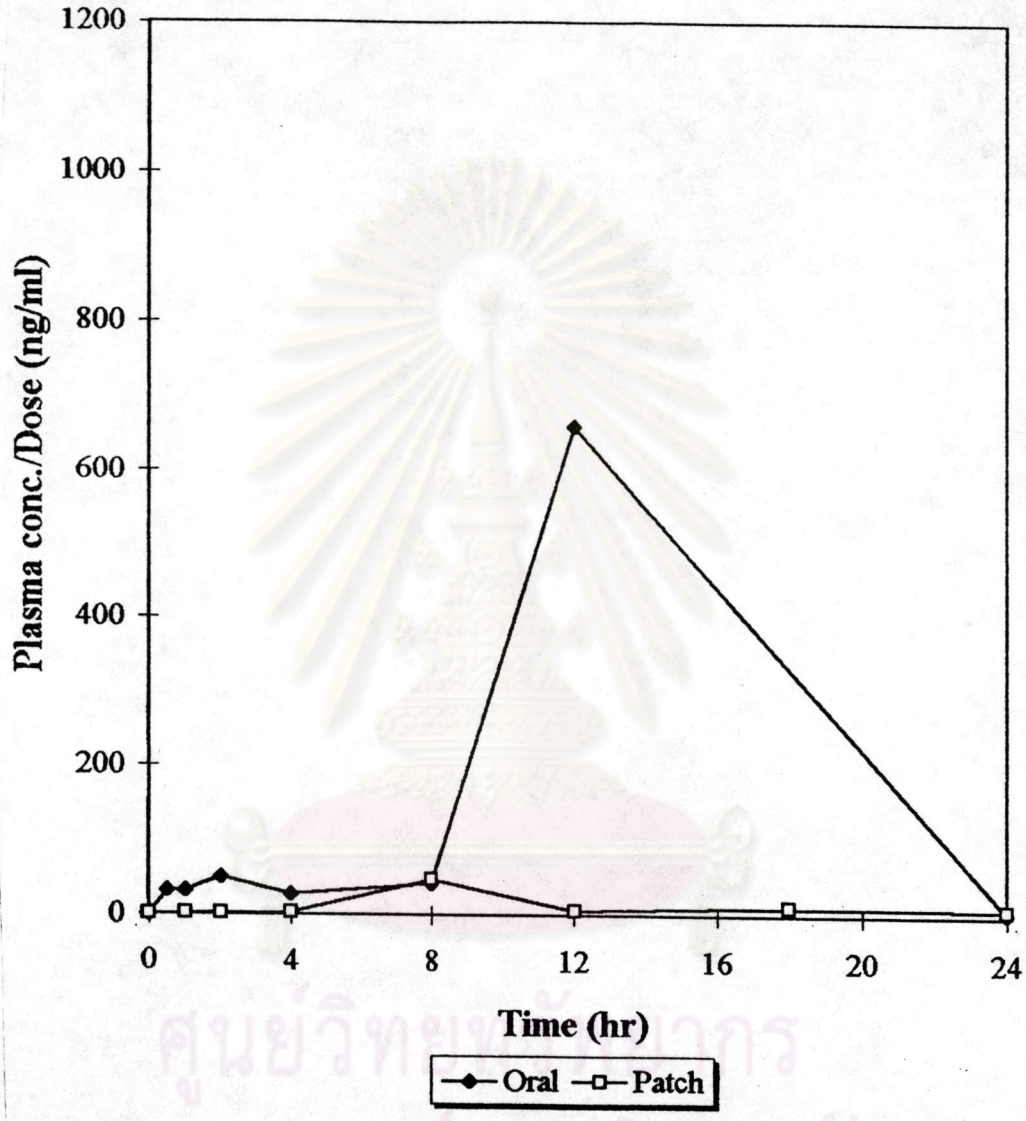


Figure 27 Normalized plasma concentration-time profiles of NFP from rabbit No.2

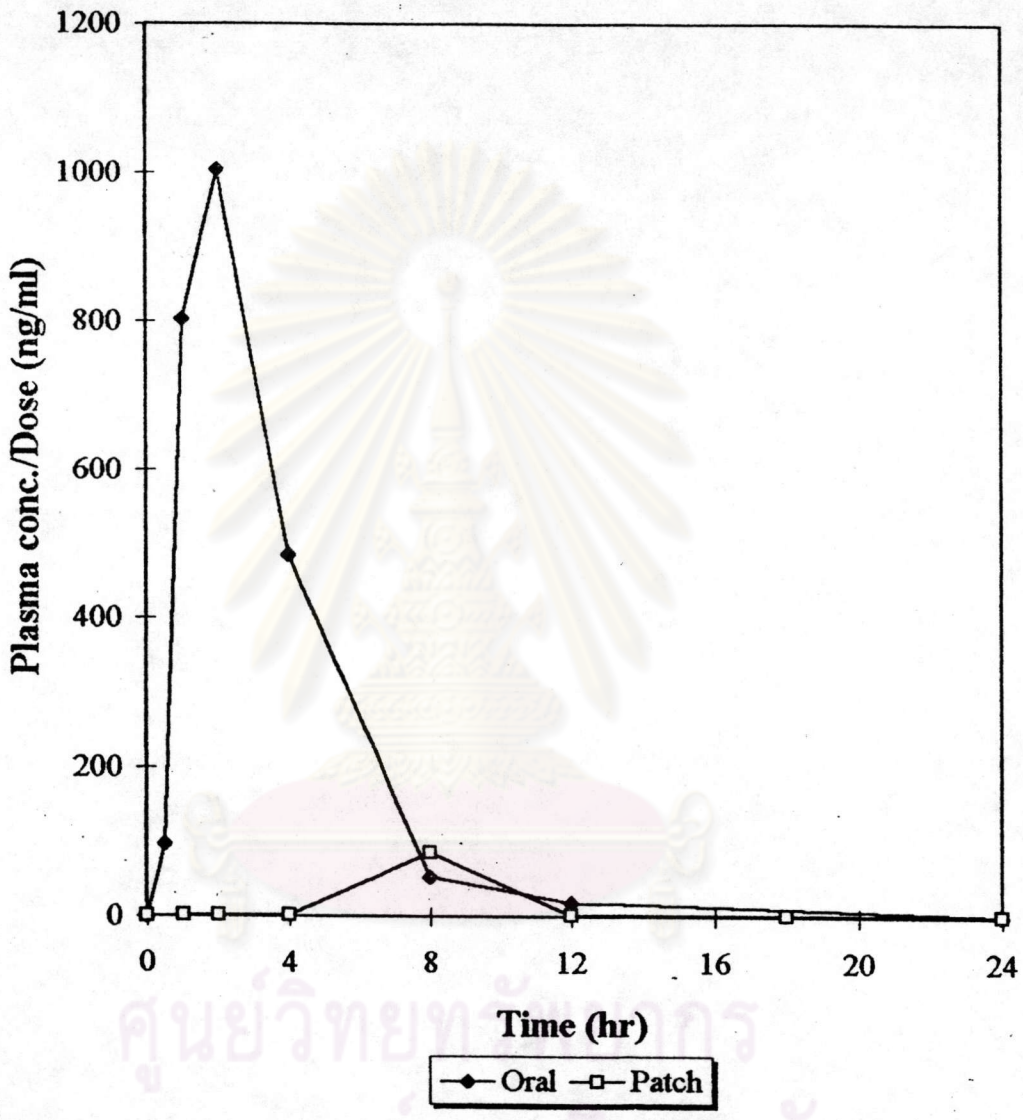


Figure 28 Normalized plasma concentration-time profiles of NFP from rabbit No.3

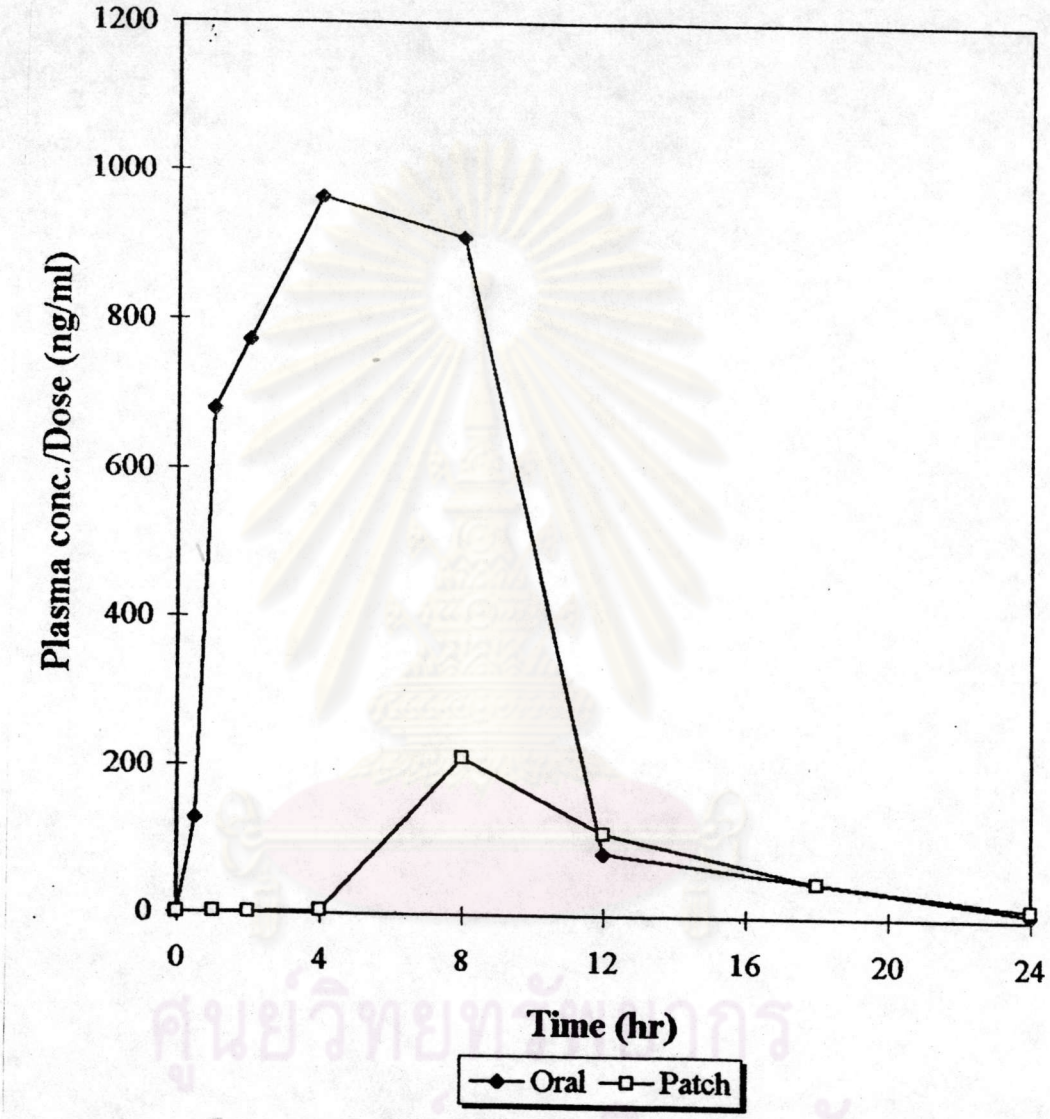


Figure 29 Normalized plasma concentration-time profiles of NFP from rabbit No.4

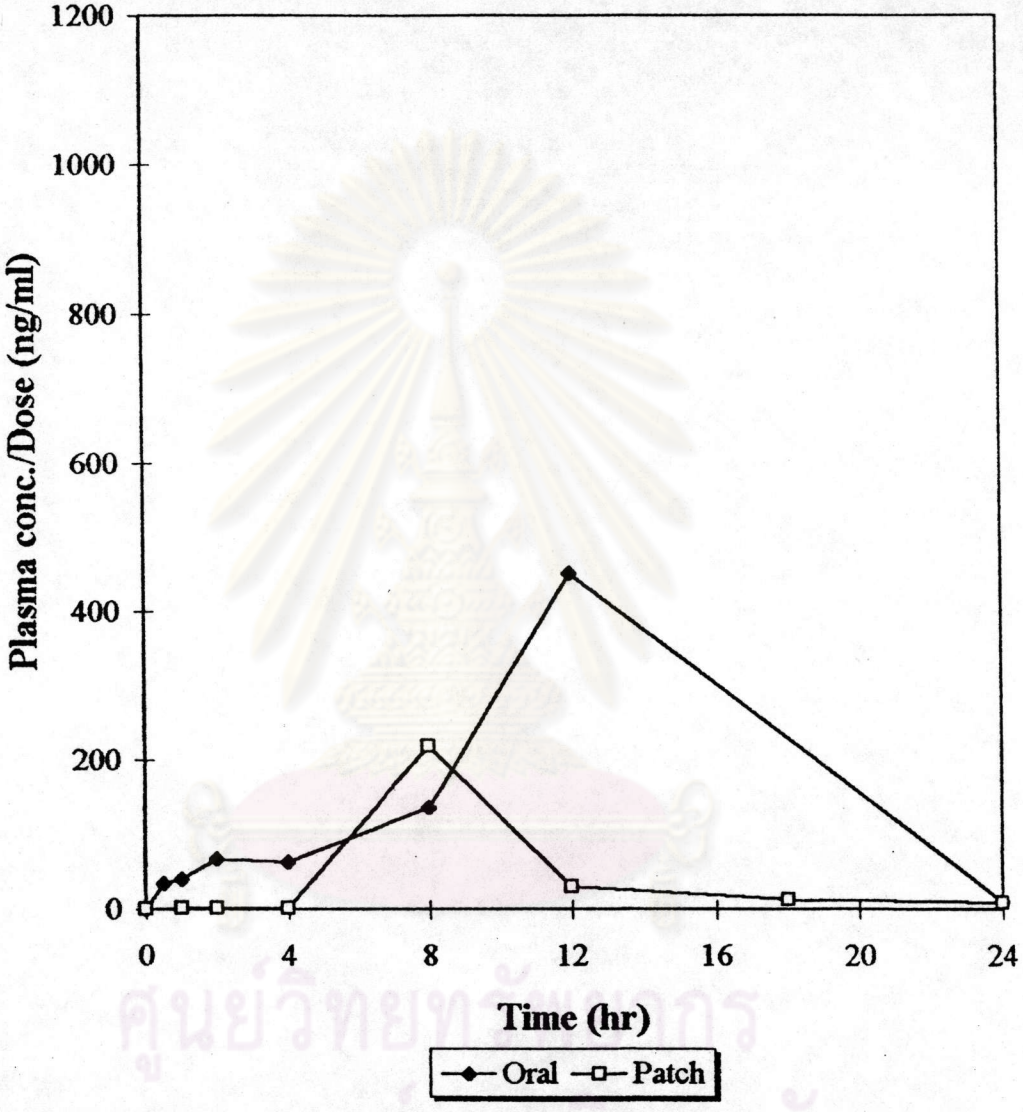


Figure 30 Normalized plasma concentration-time profiles of NFP from rabbit No.5

Table 6 NFP concentration versus time at maximum obtained from rabbit plasma sample from oral administration

Rabbit No.	Oral administration	
	t max (hr)	*Cmax (ng/ml)*
1	0.5	1108.1
2	12.0	659.9
3	2.0	1003.9
4	4.0	963.7
5	12.0	451.5

Table 7 NFP concentration versus time at maximum obtained from rabbit plasma sample from transdermal patch administration

Rabbit No.	Patch administration	
	t max (hr)	*Cmax (ng/ml)*
1	8.0	95.5
2	8.0	47.2
3	8.0	85.5
4	8.0	212.6
5	8.0	218.3

Table 8 The normalized area under the plasma concentration-time (t 0-24 hr) curve (AUC $|_0^{24}$) from oral and transdermal patch administrations

Rabbit No.	AUC (ng hr/ml)		Normalized AUC (ng hr/ml)	
	Oral	*Patch*	*Oral*	*Patch*
1	32977	36311	3298	1157
2	56743	10103	5674	322
3	39782	14283	3978	372
4	89969	67024	8997	1746
5	45505	39109	4551	1137

***In vitro - In vivo* relationship**

The normalized area under the profiles of NFP concentration released from the patch per diffusion volume at various time intervals ($AUC\Big|_0^{24}$) from *in vitro* study were highly statistical significant difference from *in vivo* study ($p < 0.001$), data was shown in Table 9

Table 9 Unpaired t-test of normalized $AUC\Big|_0^{24}$ for *in vitro* and *in vivo* evaluation.

Sample No.	Normalized AUC	
	In vitro (ng hr/ml)	*In vivo (ng hr/ml)*
1	10476	1157
2	13363	322
3		372
4		1746
5		1137
Average	11919.5	946.8
Variance	4167384.5	359962.7
n	2	5

Calculated t-value 7.738

t (5,0.05) = 2.571