CHAPTER III
RESULTS

The results of studied will be summarized as follows :

1. Analytical quantitation of nicotine

1.1 Spectrophotometric analysis of nicotine

1.2 HPLC analysis of nicotine

2. Preformulation of drug reservoir for nicotine TDS

A. Non-aqueous base preformulation

1. Effect of solvents/vehicles on stability of nicotine
2. Effect of solvents/vehicles on the release and skin permeation of nicotine

B. Aqueous base preformulation

1. Study the effect of pH on preformulation of nicotine TDS
   1.1 Effect of pH on partition coefficient
   1.2 Effect of pH on stability of nicotine
   1.3 Effect of pH on the release and skin permeation of nicotine
2. Study the effect of antioxidant/chelating agent on stability of nicotine

3. Formulations of nicotine TDS

4. Evaluation of nicotine TDS formulations

4.1 Physical characteristices evaluation of nicotine TDS formulations

4.2 *In-vitro* evaluation of nicotine TDS formulations

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

1. Analytical Quantitation of Nicotine

1.1 Spectrophotometric Analysis of Nicotine

The UV scanning for maximum absorption wavelength of nicotine was detected at the wavelength of 260 nm as shown in Figure 16. Thus, in this research study the quantitative analysis of nicotine by spectophotometric and HPLC were performed at the wavelength of 260 nm.

The calibration curve of nicotine was plotted between the concentration of drug as a fuction of absorbance. In Table 6 showed a concentration of nicotine in various pH values of phosphate buffer solutions versus its absorbance. A typical calibration plot, showed a linear relationship between the absorbance and nicotine concentration.The calibration curve of nicotine after regression analysis is illustrated in Appendices i-vi.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

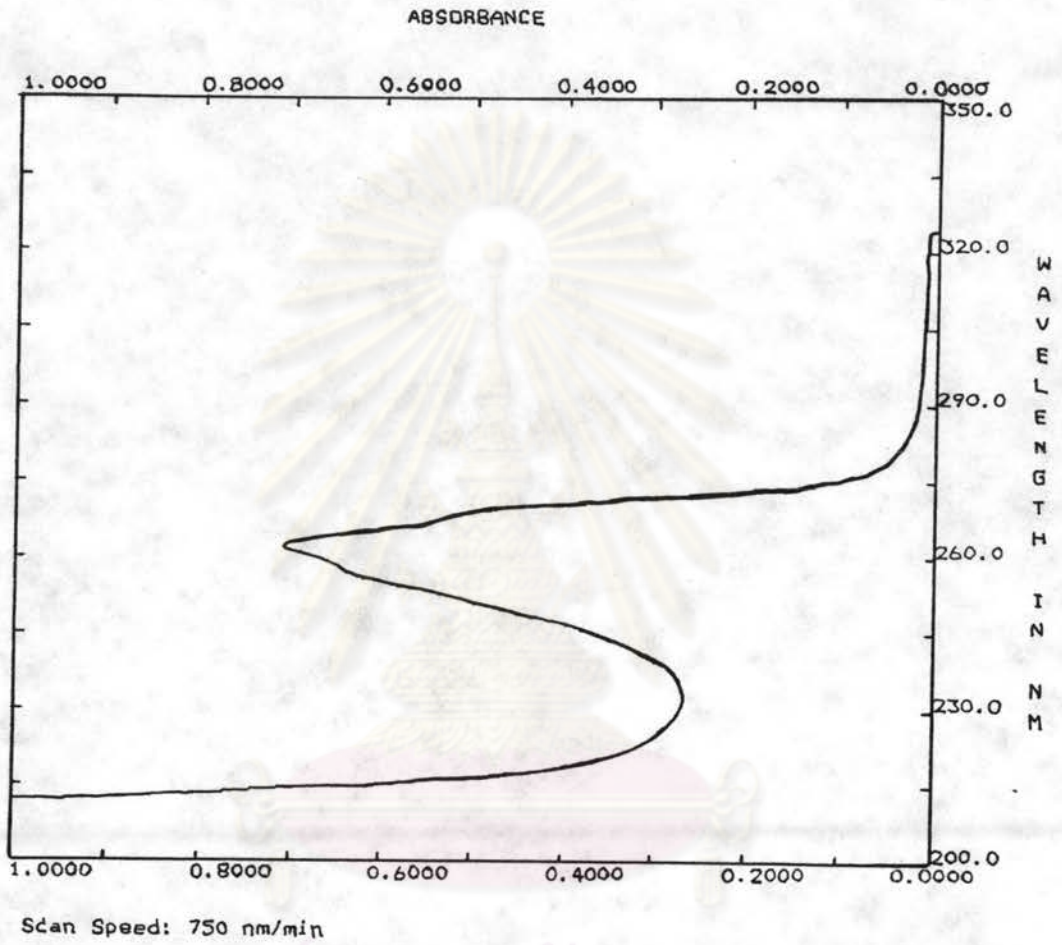


Figure 16 UV scanning curve of 40 µg/ml nicotine showing maximum absorbance at 260 nm.

Table 6 : Absorbance of nicotine in phosphate buffer at 260 nm by UV Spectrophotometry.

Conc. of nicotine (µg/ml)	Absorbance at 260 nm*					
	pH2	pH4	pH6	pH7	pH8	pH10
10	0.29	0.195	0.165	0.163	0.163	0.153
20	0.597	0.681	0.323	0.321	0.322	0.327
30	0.907	0.606	0.492	0.487	0.504	0.516
40	1.203	0.701	0.656	0.656	0.675	0.694
50	1.504	0.974	0.831	0.828	0.842	0.882

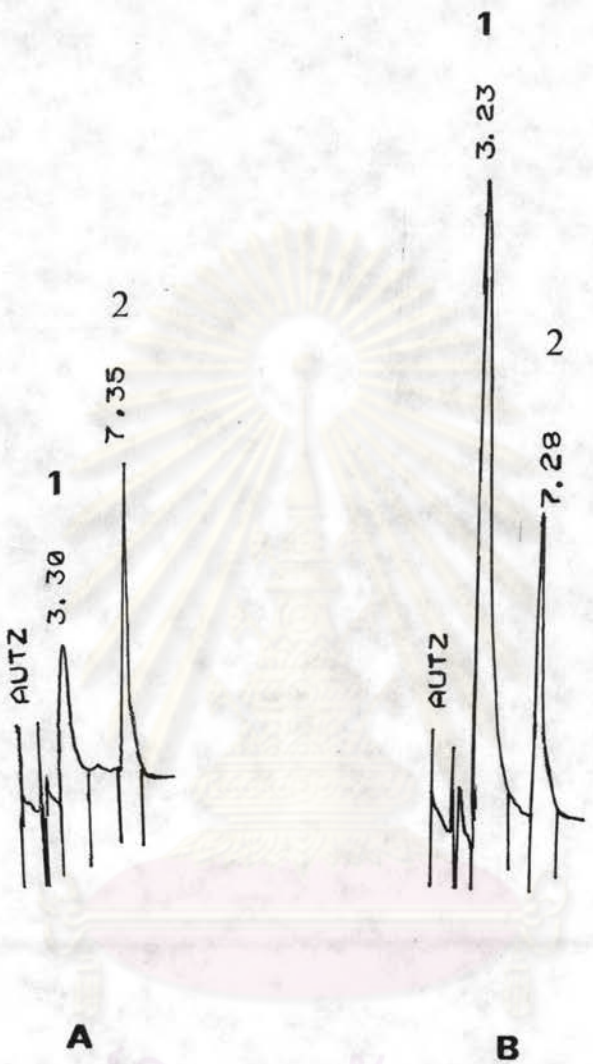
*Average of three determinations

1.2 HPLC Analysis of Nicotine

Nicotine was analyzed by reversed phase HPLC and the design chromatographic conditions were previously mentioned before. The run time per sample was within 10 minutes. Chromatogram of HPLC as shown in Figure 17 presented the good resolution between the drug and the internal strandard.

The calibration curve of nicotine was plotted by the ratio of peak area of nicotine and internal standard as a function of the nicotine concentration. The calibration plot (in Figure 18) showed a linear relationship with the good correlation coefficient. For the calculate of the concentration of nicotine in *in-vitro* evaluation, the calibration was repeated every course of the analysis.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 17 High performance liquid chromatogram of nicotine and dexamethasone (internal standard) at 260 nm [1; nicotine (A) 5.0 µg/ml (B) 40.0 µg/ml 2; dexamethasone (A) and (B) 5.0 µg/ml].

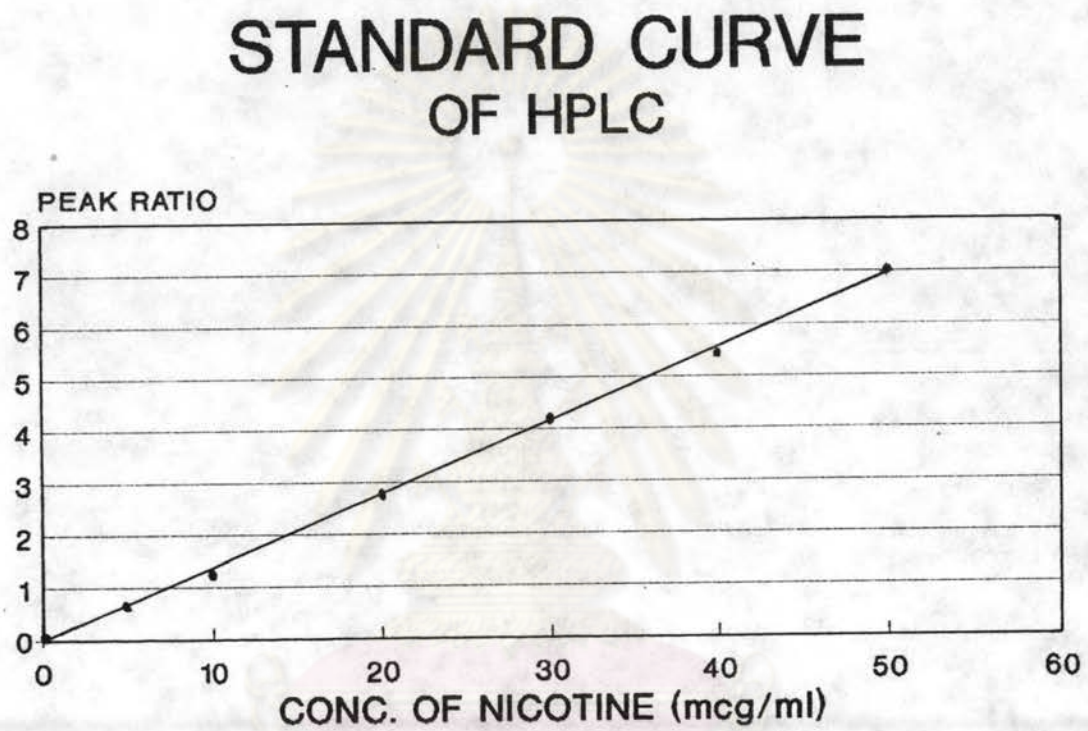


Figure 18 Calibration curve of nicotine-internal standard peak area ratio as a function of nicotine concentration range of 5-50 μg/ml.

2. Preformulation of Drug Reservoir for Nicotine TDS

A. Non-aqueous Base Preformulation

1. Study the Effect of Solvents/Vehicles on Stability of Nicotine

This part studied the effect of solvents/vehicles on the stability of nicotine. Nicotine base was added to various vehicles such as polyethylene glycol 400, propylene glycol, glycerin, mineral oil, silicone oil and simethicone, at 5% w/w concentration. The accelerated reactions were kept at 45°C for 4 weeks. The results in color change were compared and summarized in Table 7 and Figure 19.

Table 7 Degree of intensity for color change of 5 % w/w nicotine in various vehicles after keeping at 45°C for 4 weeks.

Vehicles	Color change
PEG 400	+5
propylene glycol	+4
glycerin	+4
mineral oil	+1
silicone oil	+2
simethicone	+3

(+) showed a degree of intensity of color, (n = 3)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 19 Physical stability of 5 % w/w nicotine in various vehicles after keeping at 45°C for 4 weeks.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2. Effect of Solvents/Vehicles on the Release and Skin Permeation of Nicotine Through Membrane, Adhesive and Skin.

Studies the influence of solvents/vehicles on the *in-vitro* release and skin permeation of the drug through membrane, adhesive and skin were carrier out. The release and skin permeation data of nicotine in various vehicles are presented in Appendices x-xv. The average cumulative amount of nicotine release and skin permeations are given in Tables 8-9.

The drug release with the same concentration from different vehicles does not comprise the same release rate. A typical release time profiles depicting the effect of vehicles are shown in Figure 20. Figure 21- 23 showed skin permeation time profiles which obtained from correlation between Q_s versus time, the logarithm of remained Q_s versus time and Q_s versus square root of time, respectively.

Table 8 Average cumulative release of nicotine per surface area (mg/cm^2) from nicotine-TDS patch containing 5 % w/w nicotine (2.5 mg/cm^2) in various vehicles (n=3).

Time (hrs)	Cumulative released (mg/cm^2) ± SD					
	PG	PEG 400	glycerin	mineral oil	silicone oil	simethicone
0	0	0	0	0	0	0
1	0.0385 ± 0.0026	0.1674 ± 0.0066	0.3822 ± 0.0534	0.5579 ± 0.0313	0.8464 ± 0.0605	0.8169 ± 0.0065
2	0.0595 ± 0.0023	0.2296 ± 0.0091	0.4819 ± 0.0405	0.7783 ± 0.0379	0.9922 ± 0.0289	1.0635 ± 0.0283
3	0.0791 ± 0.0044	0.2868 ± 0.0205	0.5404 ± 0.0319	0.8719 ± 0.0159	1.148 ± 0.0248	1.1683 ± 0.035
4	0.0950 ± 0.0042	0.3323 ± 0.0223	0.6251 ± 0.0445	1.0449 ± 0.0044	1.3687 ± 0.0261	1.3215 ± 0.0463
6	0.1323 ± 0.0092	0.4231 ± 0.0069	0.7019 ± 0.0465	1.2305 ± 0.0864	1.5779 ± 0.0175	1.5541 ± 0.0464
8	0.1676 ± 0.0064	0.5089 ± 0.0211	0.7849 ± 0.0392	1.4827 ± 0.0356	1.7709 ± 0.0386	1.7395 ± 0.0415
10	0.1977 ± 0.0105	0.5942 ± 0.0262	0.8976 ± 0.0550	1.7047 ± 0.0518	1.8932 ± 0.0489	1.9521 ± 0.0361
12	0.2317 ± 0.0111	0.6501 ± 0.0106	0.9496 ± 0.0532	1.7474 ± 0.0916	2.0249 ± 0.0237	2.0472 ± 0.0259

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 9 Average cumulative skin permeation of nicotine per surface area (mg/cm^2) from nicotine-TDS patch containing 5 % w/w nicotine (2.5 mg/cm^2) in various vehicles (n=3).

Time (hrs)	Cumulative skin permeation (mg/cm^2) ± SD					
	PG	PEG400	glycerin	mineral oil	silicone oil	simethicone
0	0	0	0	0	0	0
1	0.0035 ± 0.0012	0.0161 ± 0.0034	0.0119 ± 0.0002	0.0557 ± 0.010	0.1552 ± 0.005	0.0805 ± 0.0022
2	0.0062 ± 0.0012	0.0515 ± 0.0045	0.0256 ± 0.0024	0.1233 ± 0.0238	0.2779 ± 0.026	0.1901 ± 0.0015
3	0.0127 ± 0.0004	0.0800 ± 0.0121	0.0609 ± 0.0205	0.205 ± 0.0141	0.4211 ± 0.0317	0.3108 ± 0.0031
4	0.0209 ± 0.0019	0.1036 ± 0.0102	0.1012 ± 0.0222	0.2803 ± 0.0187	0.5448 ± 0.0033	0.3768 ± 0.0043
6	0.0358 ± 0.0028	0.1646 ± 0.0102	0.1613 ± 0.0075	0.3814 ± 0.0205	0.6976 ± 0.0583	0.4990 ± 0.0073
8	0.0615 ± .0023	0.2058 ± 0.0143	0.2405 ± 0.0088	0.5529 ± 0.0662	0.7990 ± 0.0284	0.7039 ± 0.0190
10	0.0786 ± 0.0033	0.2499 ± 0.6067	0.3057 ± 0.0036	0.7695 ± 0.0045	0.8724 ± 0.0269	0.8127 ± 0.0036
12	0.0986 ± 0.0051	0.3312 ± 0.0086	0.3742 ± 0.0034	0.9027 ± 0.0173	0.9411 ± 0.0265	0.9189 ± 0.0079

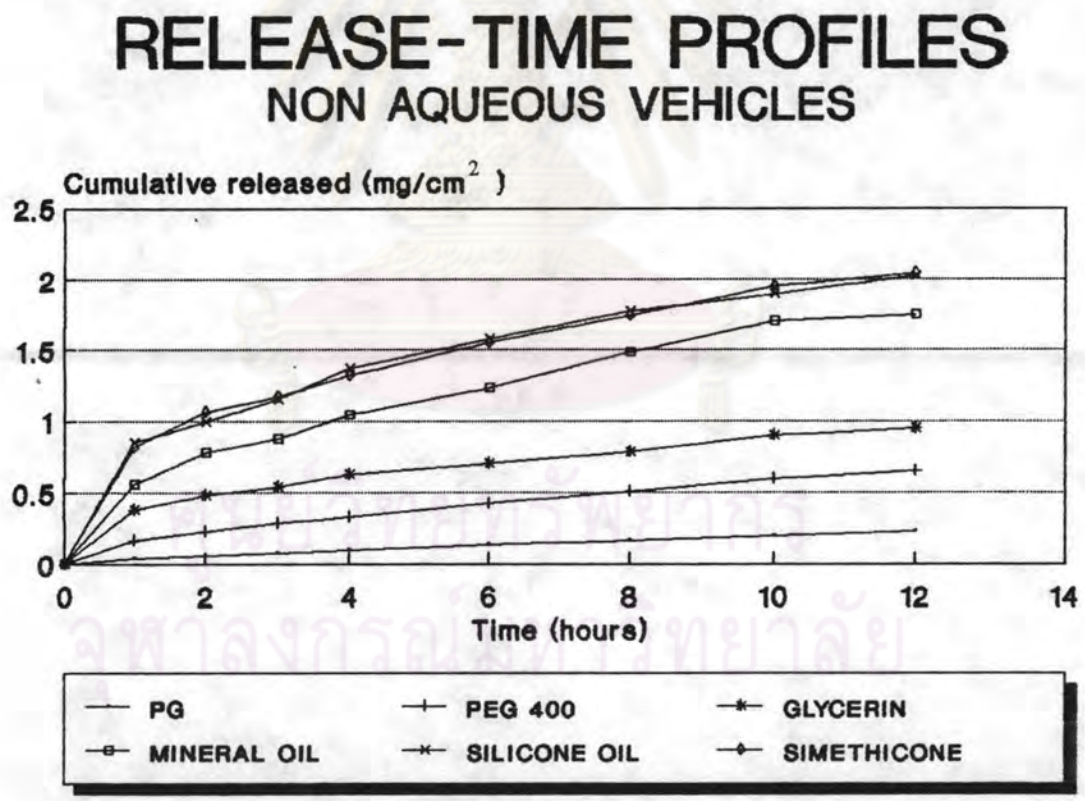


Figure 20 Average release-time profiles of nicotine-TDS patch containing 5% w/w nicotine in various vehicles (n=3).

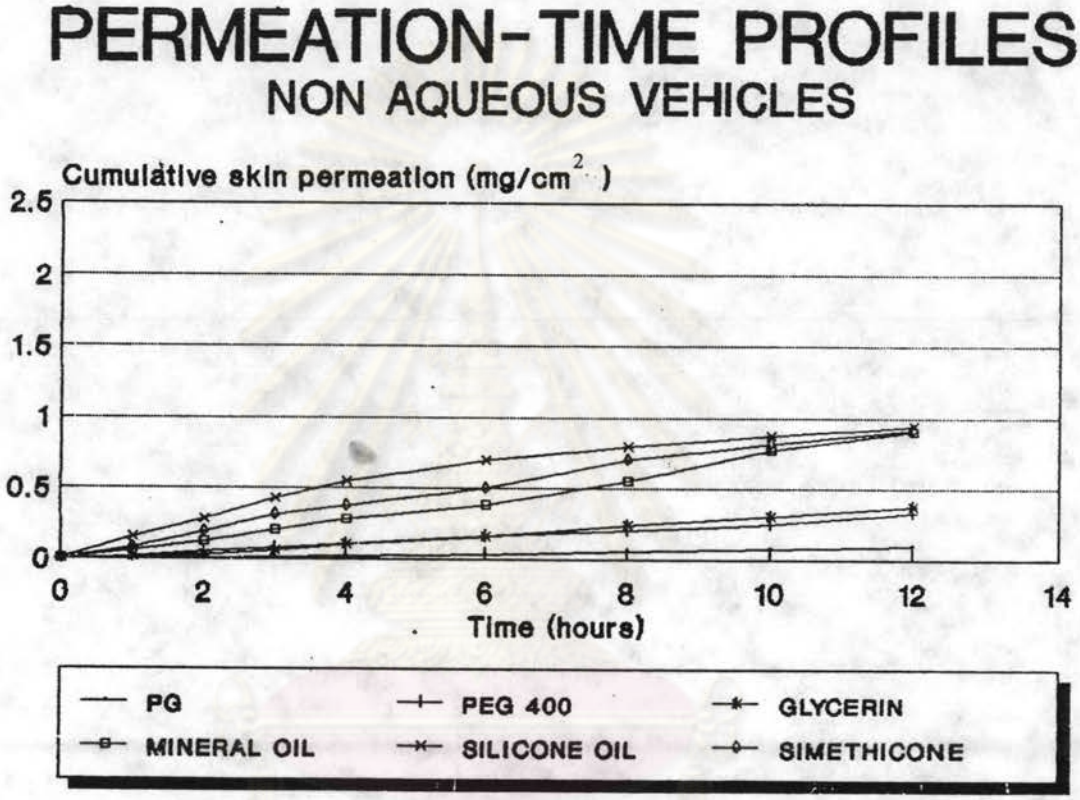


Figure 21 Average skin permeation-time profiles of nicotine-TDS patch containing 5% w/w nicotine in various vehicles (n=3).

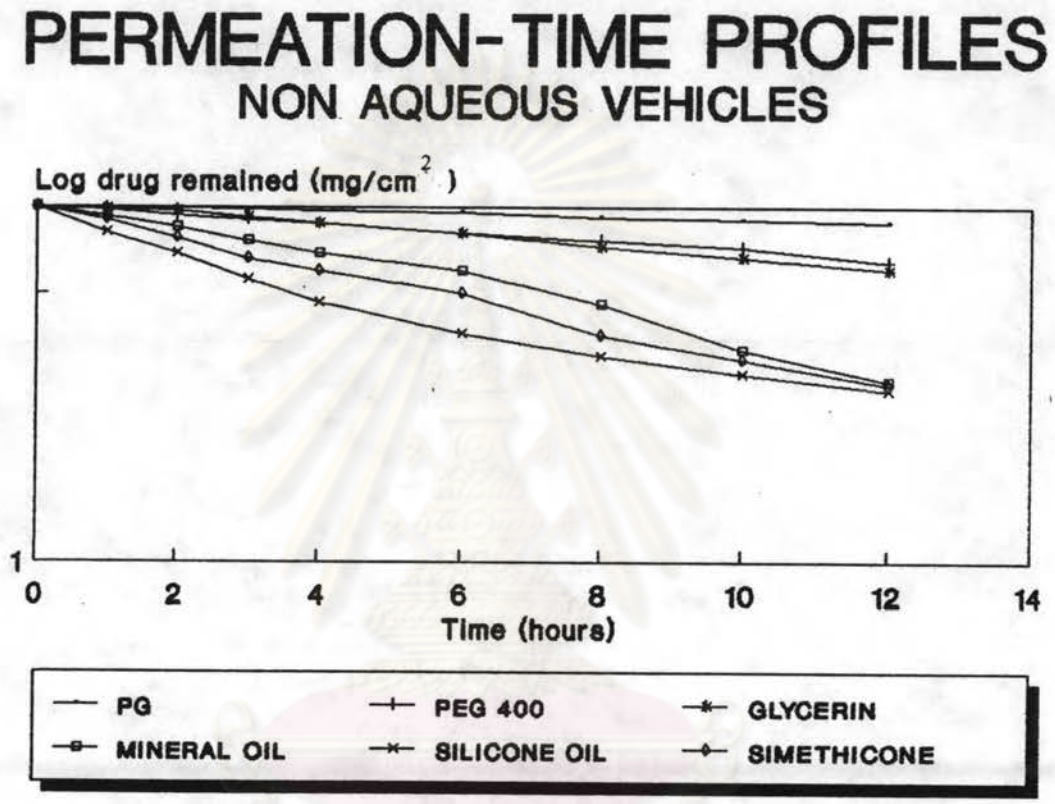


Figure 22 Average logarithm of drug remaining-time profiles of nicotine-TDS patch containing 5 % w/w nicotine in various vehicles (n=3).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

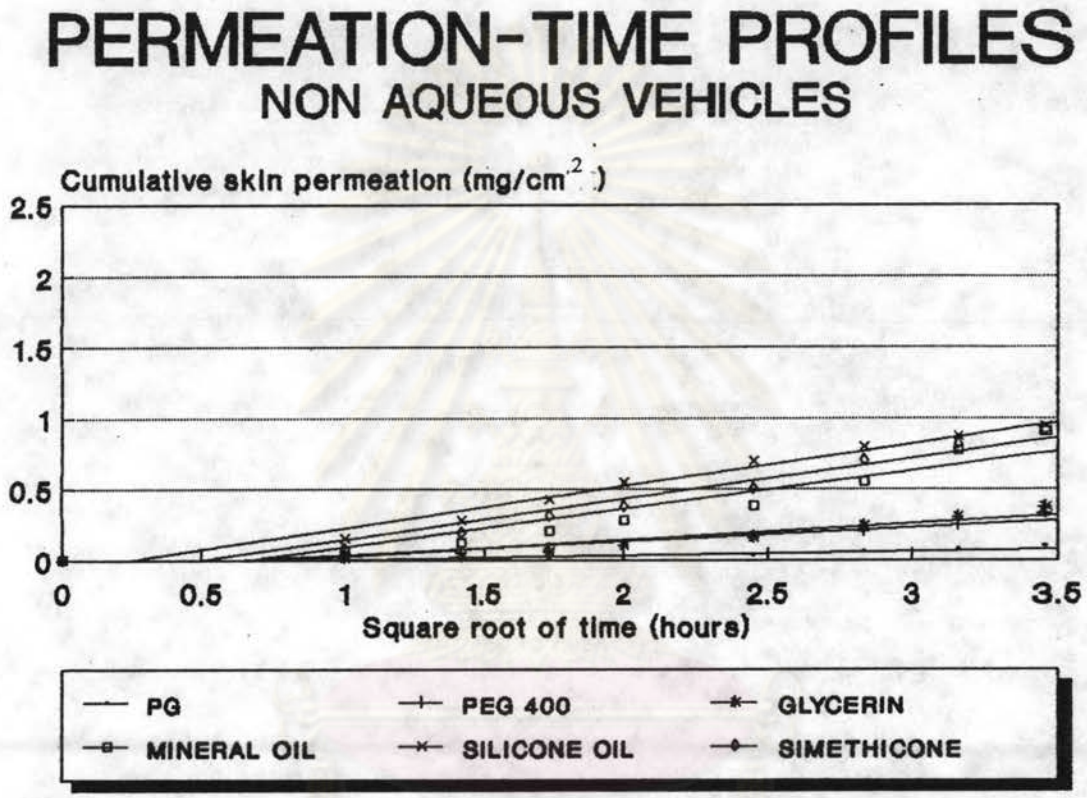


Figure 23 Average skin permeation-square root of time profiles of nicotine-TDS patch containing 5 % w/w nicotine in various vehicles (n=3).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

B. Aqueous Base Preformulation

1. Study the Effect of pH Values on Preformulation of Nicotine-TDS

1.1 Effect of pH Values on Partition Coefficient

Octanol/buffer partition coefficients were determined using 0.1 M phosphate buffers. The results of octanol/buffer partition coefficient versus pH are shown in Table 10 and apparent partition coefficients as a function of pH are shown in Figure 24.

The apparent partition coefficient describes the partitioning characteristics of a molecule without separating the effects of drug association or dissociation. Determination of the true partition coefficient, which considers only the partitioning of the unionized base between the two phases also makes it possible to obtain information on ion pair formation. In order to study the effect of dissociation on the partitioning of nicotine, a quantitative relationship between the apparent partition coefficient (K), the true partition coefficient (K_u), and pH was sought. By this approach, adherence to the pH partition hypothesis could be tested (Oakley and Swarbrick, 1987).

Table 10 The results of n-octanol/buffer partiton coefficient as a function of pH values.

pH	Conc. of nicotine in buffer(μg/ml)			Conc. of nicotine in octanol(μg/ml)	Apparent partition coeff. of nicotine (K)
	A	B	x		
6	48.4587	49.0095	48.7343	1.2657	0.0259
7	29.3393	28.4985	28.9189	21.0811	0.7289
8	8.2206	8.2796	8.2501	42.7499	5.0605
10	5.6493	5.4301	5.5397	44.4603	8.0258

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

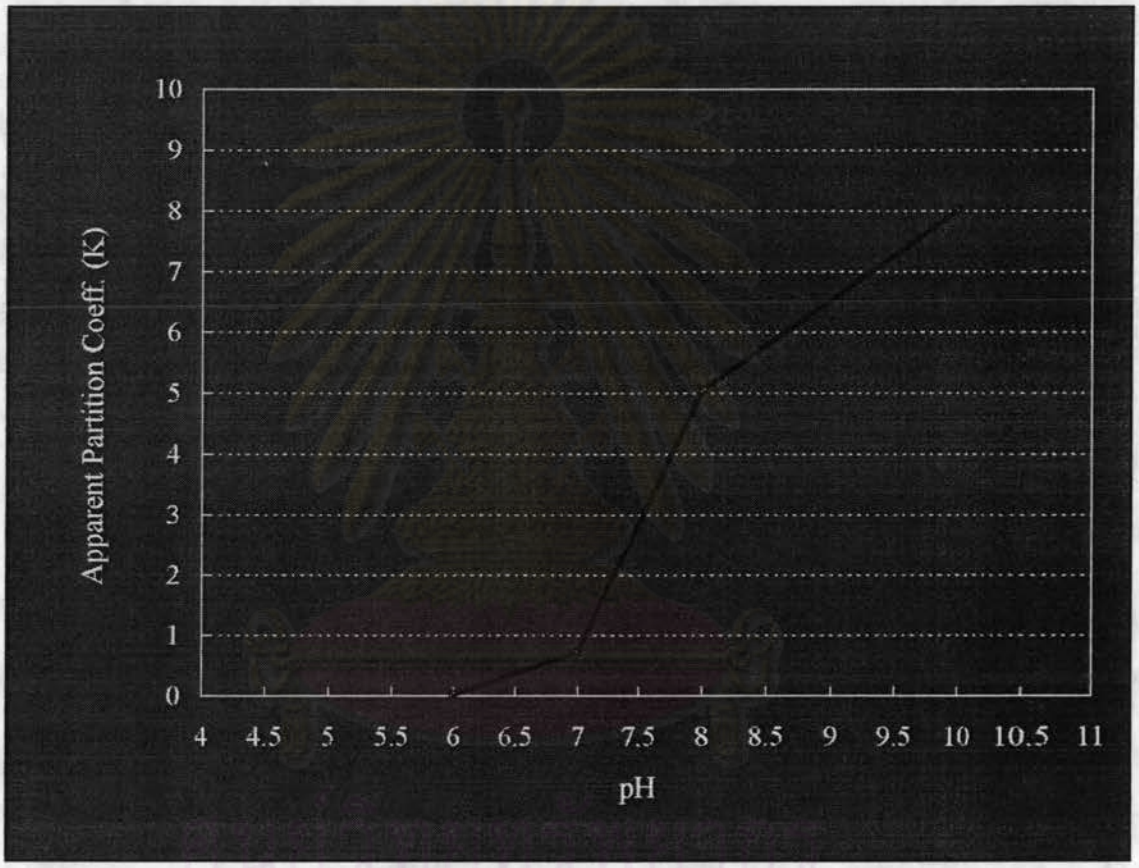


Figure 24 Apparent partition coefficient as a function of pH values.

The second dissociation of a diacidic base (or dissociation of a mono-acidic base) can be described by :

$$BH^+ + H_2O \longrightarrow B + H_3O^+$$

$$K_{a,2} = \frac{[B]\,[H_3O^+]}{[BH^+]} \quad \text{.....(13)}$$

$K_{a,2}$ is the second dissocoation constant

The true partition coefficient (K_u) of the unionized free base is gives by:

$$K_u = \frac{[B]_o}{[B]_a} \quad \text{.....(14)}$$

Where, K_u is the true partition coefficient, $[B]_o$ is concentration of drug in organic phase $[B]_a$ is concentration of drug in water phase

The apparent partition coefficient (K) at any pH values is given by:

$$K = \frac{[B]_o}{[B]_a + [BH^+]_a + [BH^{++}]_a} \quad \text{.....(15)}$$

K is the apparent partition coefficient

According to the model of Irwin and Li Wan Po (1979), modified by Oakley and Swarbrick (1987), the true partition coefficients of nicotine was calculated from the measured apparent coefficients. The equation used in the pH range of 4.0-7.5 is

$$K\,(1 + K_a/[H_3O^+]\,) = K_{ip} + K_u(K_a/[H_3O^+]) \quad \text{.....(16)}$$

Where K is the apparent partition coefficient, K_{ip} denotes the partition coefficient of the ion pair, K_u represents the true partition coefficient, and K_a is the dissociation constant

For nicotine the $K_{a,2}$ value was used as K_a By plotting $K\,(1+K_a/[H_3O^+])$ versus $K_a/[H_3O^+]$, a straight line is obtained whose slope corresponds

to the true partition coefficient and the intercept to the ion pair partition coefficient (Figure 25).

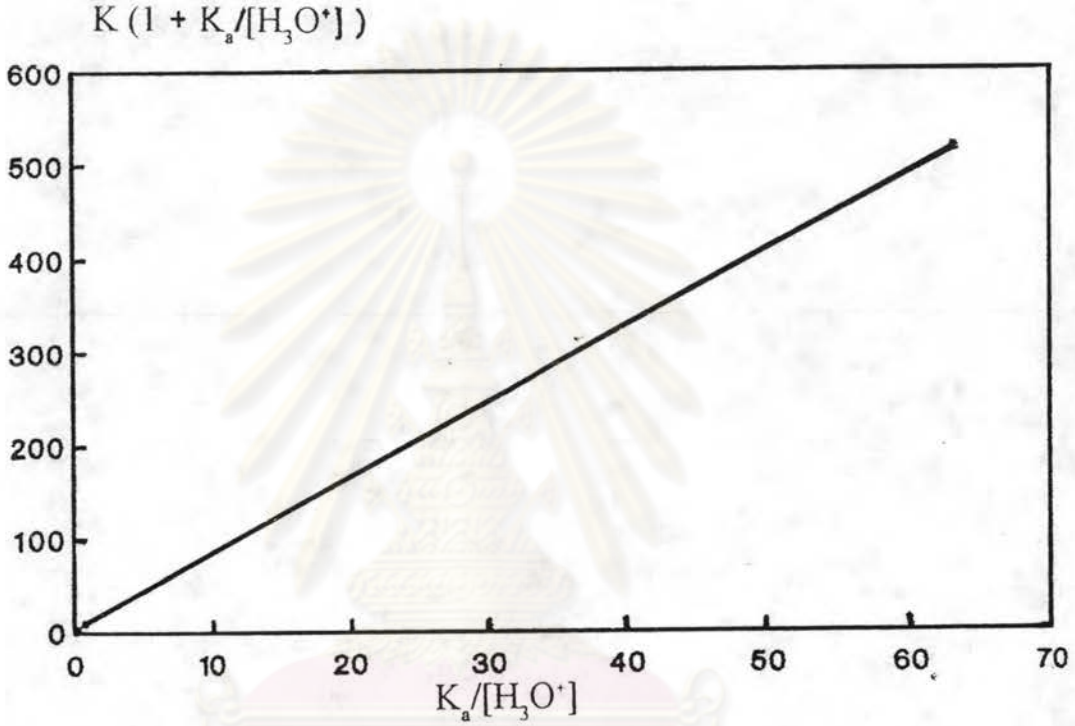


Figure 25 Determination of K_u and K_{ip} of nicotine in octanol : phosphate buffer system.

In order to confirm this result, the adherence to the pH partition theory of n-octanol/buffer distribution of the drugs studied was verified, assuming that the concentration of the dication (BH^{++}) is insignificant, substitution of equation 14 and 15 into equation 13 and taking log yields:

$$\log (K/K_u) = \log (K_{a,2}/K_{a,2}+[H_3O^+]) \quad \text{.....(17)}$$

Examination of Equation 17 shows that if $k_{a,2} >> [H_3O^+]$, Then:

$$\log (K/K_u) \longrightarrow 0 \quad \text{.....(18)}$$

If $K_{a,2} << [H_3O^+]$, then :

$$\log (K/K_u) \longrightarrow pH - pK_{a.2} \quad \text{.....(19)}$$

By using Equation 17, it is possible to draw the curve describing the theoretical profile of variation of log (K/K_u) as a function of pH. In Figure 26 the variations of log (K/K_u) found with nicotine, compared with the theoretical profiles,are presented versus pH (Oakley and Swarbrick, 1987; Thassu and Vyas, 1993; Irwin and Li Wan Po; 1979).

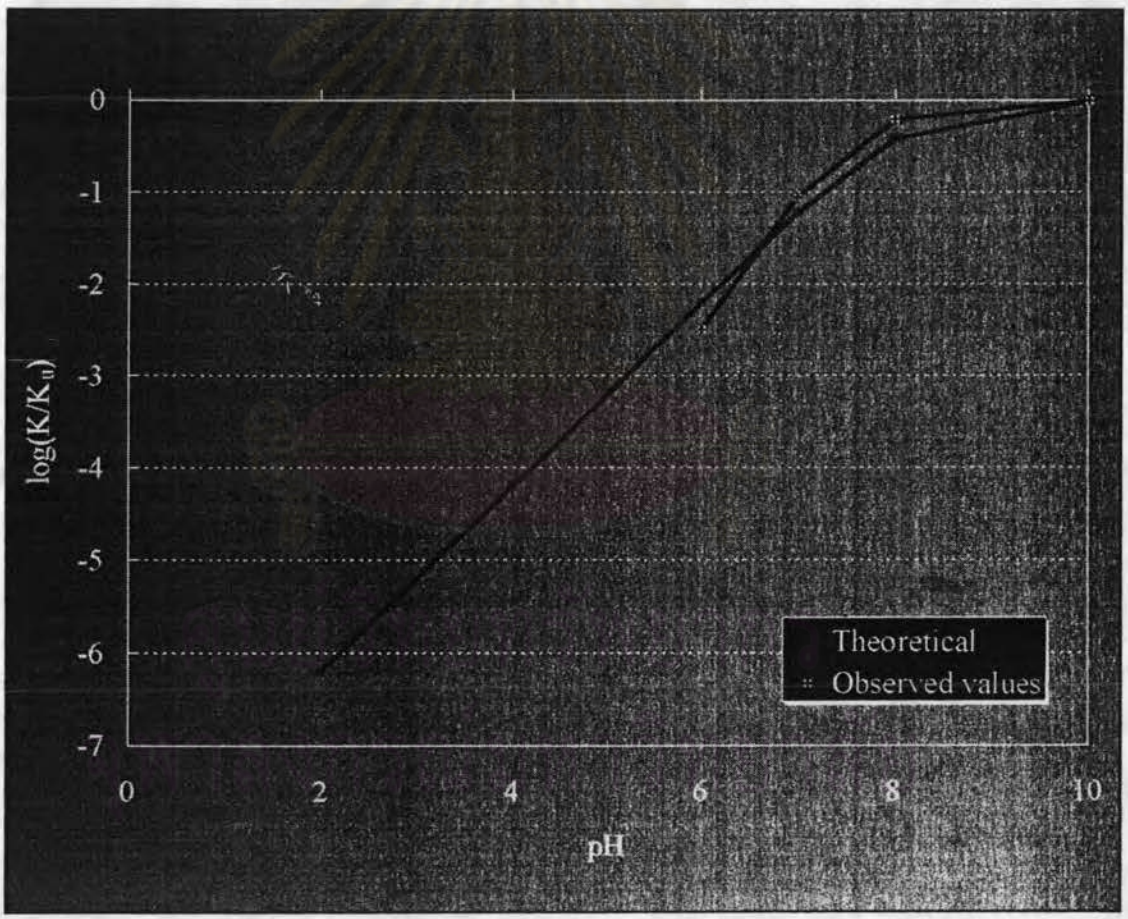


Figure 26 Variation of log (K/K_u) as a function of pH values.

1.2 Effect of pH values on Stability of Nicotine

Because of the complex mixtures that may occur in dosage forms, many kinds of drug decomposition reactions are possible. Nicotine is a tertiary amine composed of a pyridine and a pyrrolidine ring and turns brown on exposure to air or light (Robinson, Balter and Schwartz, 1992). This part studied the effect of pH values on the stability of nicotine solution. In Table 11 and Figure 27 showed physical characteristics change of 5 % w/w nicotine solution at pH values of 2, 4, 6.5, 8.5 and 10.5 after keeping at 45°C upto 4 weeks. Table 12 showed percentage recovered versus pH and Figure 28 showed typical accelerated pH stability profile on preformulation studies of 5 % w/w nicotine solution at pH values of 2, 4, 6.5, 8.5 and 10.5 after keeping at 45°C for 4 weeks analysis by HPLC method.

Table 11 Physical characteristics change of nicotine in aqueous solution at pH range of 2-10.5.

Time	Color change at pH				
(days)	2	4	6.5	8.5	10.5
0	+1	+1	+1	+1	+1
7	+2	+2	+2	+1	+1
14	+3	+3	+3	+1	+1
21	+4	+4	+4	+2	+2
28	+5	+5	+5	+2	+2

Remark : (+) Showed a degree of intensity of color, (n = 3)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 27 Color change of nicotine aqueous solutions at pH values of 2, 4, 6.5, 8.5 and 10.5 after keeping at 45°C for 4 weeks.

Table 12 Percentage recover of nicotine in pH 2-10.5 phosphate buffer solutions after keeping at 45° C for 4 weeks analysed by HPLC method.

pH	Percentage recovered
2	88.8814
4	89.3838
6.5	90.0058
8.5	94.6421
10.5	94.8824

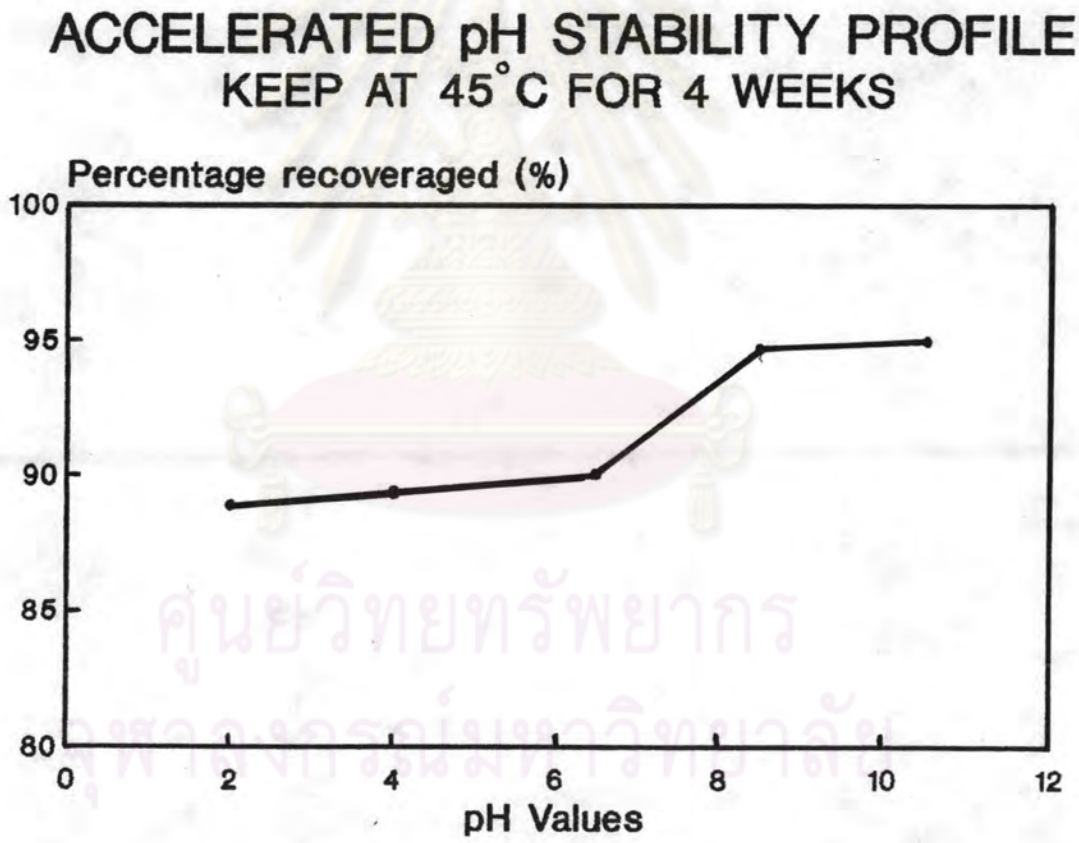


Figure 28 Typical accelerated pH stability profile on preformulation studies of nicotine in pH 2-10.5 phosphate buffer solutions after keeping at 45°C for 4 weeks.

1.3 Effect of pH Values on the Release and Skin Permeation of Nicotine Through Membrane, Adhesive and Skin

1.3.1 Effect of pH Values on Release of Nicotine

From the stability of nicotine in pH 2-10.5 buffer solutions revealed that at pH above 6.5 nicotine was more stable than pH below 6.5. Thus, these three pH values of the nicotine aqueous solution (pH 6.5, 8.5 and 10.5) were selected for study the effect of pH on the release and skin permeation.

A comparision of nicotine release profiles from the three pH values of nicotine aqueous solutions revealed that increased the pH of the solution increased drug release through membrane and adhesive, however nicotine at pH 8.5 and pH 10.5 showed no significant difference in drug release characteristics. The release data of nicotine solution from the three pH values were presented in Appendices vii-ix. A typical release profile depicting the effect of increasing pH of nicotine aqueous solutions on drug release shown in Table 13 and Figure 29.

Table 13 The average cumulative amount of nicotine release and skin permeation per surface area (mg/cm^2) from nicotine-TDS patch containing 5% w/w nicotine (2.5 mg/cm^2) in aqueous vehicles with the pH's of 6.5, 8.5 and 10.5 (n=3).

Time	Cumulative released (mg/cm^2) ±SD			Skin permeation (mg/cm^2) ± SD		
(hrs)	pH 6.5	pH 8.5	pH 10.5	pH 6.5	pH 8.5	pH 10.5
0	0	0	0	0	0	0
1	0.0179 ± 0.0049	0.0291 ± 0.0024	0.0403 ± 0.007	0.0211 ± 0.0034	0.0295 ± 0.0146	0.1159 ± 0.0168
2	0.1274 ± 0.0098	0.1370 ± 0.0108	0.1447 ± 0.0279	0.0531 ± 0.0036	0.0711 ± 0.0523	0.2365 ± 0.0118
3	0.2457 ± 0.0231	0.2747 ± 0.0262	0.0230 ± 0.0193	0.0984 ± 0.0074	0.1873 ± 0.0302	0.3257 ± 0.0071
4	0.3378 ± 0.0297	0.4009 ± 0.0069	0.3781 ± 0.0353	0.1496 ± 0.0077	0.2577 ± 0.0312	0.4296 ± 0.0287
6	0.5529 ± 0.0230	0.6268 ± 0.0091	0.6661 ± 0.0431	0.2421 ± 0.0339	0.4212 ± 0.0479	0.5960 ± 0.0389
8	0.7049 ± 0.0106	0.7977 ± 0.022	0.858 ± 0.0489	0.3563 ± 0.0149	0.5994 ± 0.0312	0.7438 ± 0.0249
10	0.9132 ± 0.0093	1.0389 ± 0.0100	1.054 ± 0.0301	0.4591 ± 0.0327	0.7543 ± 0.0464	0.8899 ± 0.0007
12	1.0142 ± 0.0166	1.2825 ± 0.0502	1.2113 ± 0.0209	0.5777 ± 0.0094	0.9164 ± 0.007	1.0937 ± 0.0504

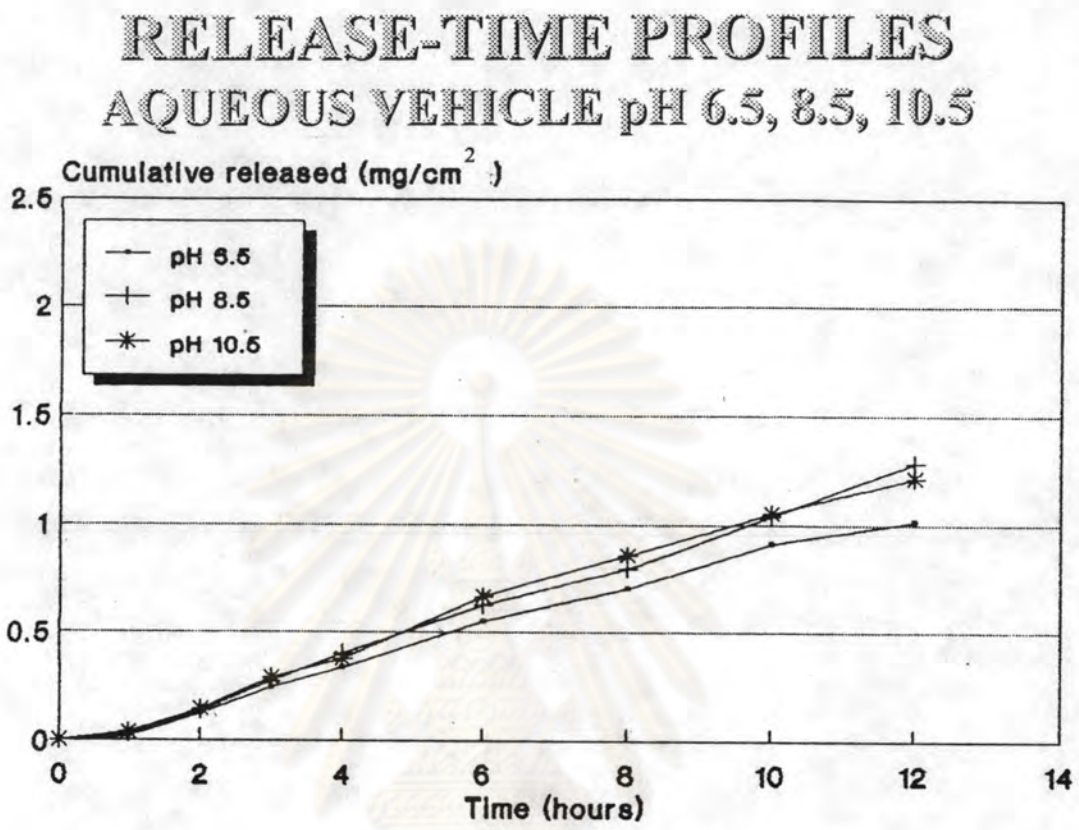


Figure 29 Average release-time profiles of nicotine-TDS patch containing 5% w/w nicotine in aqueous vehicle at pH values of 6.5, 8.5 and 10.5 (n=3).

1.3.2 Effect of pH Values on the Skin Permeation of Nicotine

The skin permeation of nicotine delivered by nicotine-TDS was investigated using the pig skin. The results of nicotine skin permeation from three different pH values in aqueous buffer solutions showed in Appendices vii-ix, Table13 and Figure 30, respectively. The results revealed that increasing pH of the solutions significantly increased drug permeation through the skin. The skin permeation time profiles indicated that the fastest drug permeation was found at pH 10.5 in contrast at pH 6.5 the slowest drug permeation was observed and at pH 8.5 the drug permeation was intermediate.

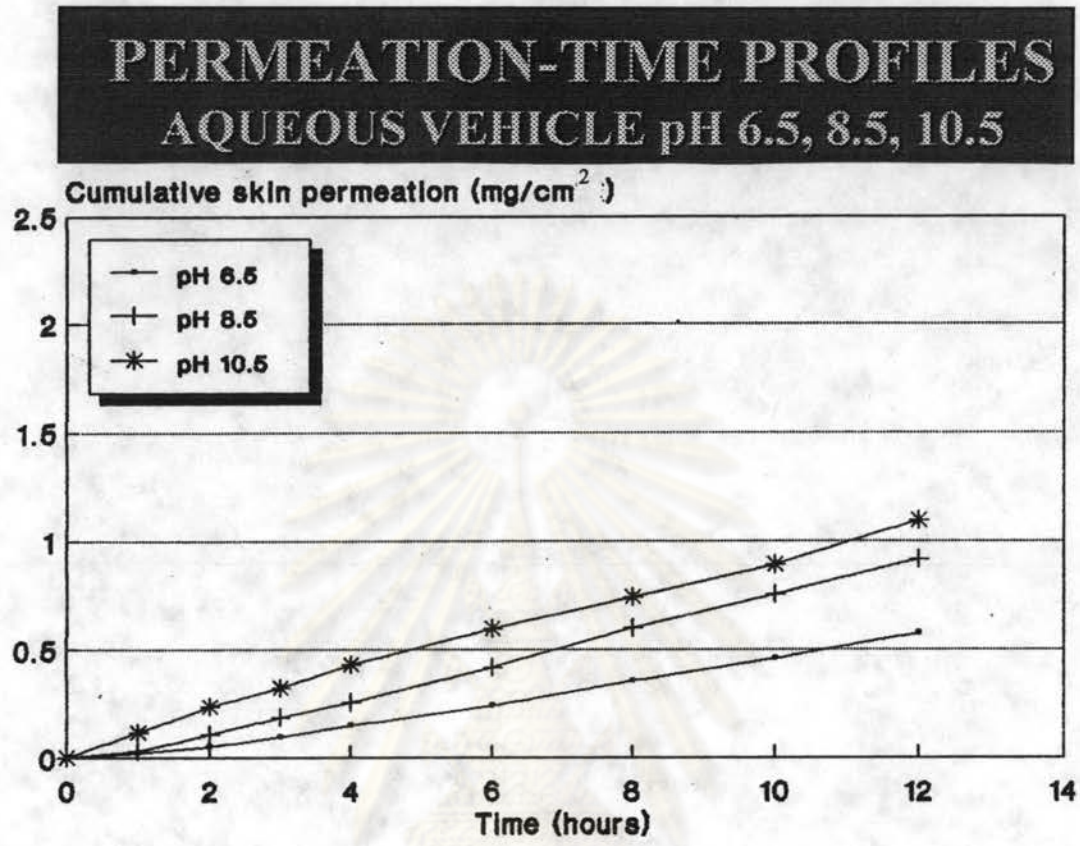


Figure 30 Skin permeation-time profiles of nicotine-TDS patch containing 5% w/w nicotine in aqueous vehicle at pH values of 6.5, 8.5 and 10.5 (n=3).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

2. Study the Effect of Antioxidant/Chelating Agent on Stability of Nicotine

Nicotine is very susceptible to oxidation. It gradually becomes brown on exposure to air, heavy metal or light. Color formation in nicotine solution is an oxidative degradation. Use of antioxidant and chelating agent may effective in increasing the stability of nicotine formulations. Several agents have been added to nicotine in attempts to inhibit the oxidative color forming reactions. Sodium sulfite and sodium EDTA possessed some stabilizing effects on color formation (Connor, K.A. and Amidon, G.L. and Stella, V.J. 1986). In this part was studied the suitable concentration of antioxidant and chelating agent for the nicotine in preparation. 0.01 - 0.05 % w/w sodium EDTA, 0.05 - 0.5 % w/w sodium sulfite and the combination of sodium EDTA and sodium sulfite were utilized to study the effect of antioxidant and chelating agent on the stability of nicotine in aqueous vehicles. Acceleration studied of antioxidant/chelating agent by keeping at 45°C for 4 weeks and results are summarized in Table 14 and Figure 31.

Table 14 Effect of sodium sulfite and sodium EDTA on the stability of nicotine in aqueous vehicle after keeping at 45°C for 4 weeks.

Experiment #	Conc. of sodium EDTA (% w/w)	Conc. of sodium sulfite (% w/w)	Color change after kept at 45 °C for 4 weeks
1	0.01	-	+4
2	0.03	-	+4
3	0.05	-	+4
4	-	0.05	+3
5	-	0.1	+3
6	-	0.5	+2
7	0.01	0.05	+3
8	0.03	0.1	+1
9	0.05	0.5	+1

(+) showed a degree of intensity of color (n = 3).

Figure 31 Physical stability of 5 % w/w nicotine in aqueous solution with antioxidant/chelating agent (according to Table 5) after keeping at 45°C for 4 weeks.

3. Formulations of Nicotine TDS

In order to fabricate nicotine transdermal drug delivery system by using solvents/vehicles, gelling agents, matrix films as drug carriers. Each preparation consisted of a fixed concentration of nicotine as 2.5 mg/cm^2.

3.1 Nicotine-mineral oil

From the previous studies, preformulation of nicotine in various solvents/vehicles indicated that mineral oil was very stable and had a high release rate. From this reason, mineral oil was selected as a solvent for further study.

Formula #1 containing 5% w/w nicotine , antioxidant used 0.1% w/w butylated hydroxyanisale (BHA) (Gont, 1990), and mineral oil qs. to 100 % w/w.

Formula #2 was developed from formula #1 but using aerosil as a gel forming. The required amount of aerosil was slowly added to mineral oil. The mixture was agitated to thoroughly mix and other additives were subsequently added. The mixture was mixed well for complete homogeneity.

Formula #3 was developed by using 20 % w/w nicotine in mineral oil adsorbed on a non woven patch that was able to blot the nicotine solution but did not bond to nicotine. The various formulations of nicotine in mineral oil are listed in Table 15.

Table 15 Formulas of nicotine-mineral oil systems.

Ingredient	Function	Formula # (% w/w)		
		1	2	3
Nicotine	active drug	5	5	20
BHA	antioxidant	0.1	0.1	0.1
Aerosil	gelling agent	-	5	-
Mineral oil qs. to	solvent	100	100	100

3.2 Nicotine-carbomer 934

From the previous studies indicated that for the effect of pH values in aqueous solution of nicotine pH 8.5 was the suitable pH that nicotine stable and higher release rate than the others. The effect of antioxidant/ chelating agent on nicotine aqueous solution indicated that 0.5% w/w sodium sulfite and 0.05% w/w sodium EDTA was shown the most effective inhibitor of color development. The concentration of carbomer 934 in the formulation was vary in the range of 0.3-1.5 % w/w. The quantities used for preparing the gel formulation are listed in Table 16.

Table 16 Formulas of nicotine-carbomer 934 systems.

Ingredient	Function	Formula # (% w/w)			
		4	5	6	7
Carbomer 934	polymer	0.3	0.5	1	1.5
Nicotine	active drug	5	5	5	5
Sodium EDTA	chelating agent	0.05	0.05	0.05	0.05
Sodium sulfite	antioxidant	0.5	0.5	0.5	0.5
Water qs. to	solvent	100	100	100	100

3.3 Nicotine-pluronic F-127

The quantities used for preparing the gel formulations are listed in Table 17.

Table 17 Formulas of nicotine-pluronic F-127 systems.

Ingredient	Function	Formula # (%w/w)		
		8	9	10
Pluronic F-127	polymer	1	5	10
Nicotine	active drug	5	5	5
Sodium EDTA	chelating agent	0.05	0.05	0.05
Sodium sulfite	antioxidant	0.5	0.5	0.5
Water qs. to	solvent	100	100	100

3.4 Nicotine-EVA copolymer

The quantities used for preparing the formulation are listed in Table 18.

Table 18 Formulas of nicotine-EVA copolymer.

Ingredient	Function	Formula #				
		11	12	13	14	15
EVAco (40%)	polymer	0.5 g	1.0 g	1.5 g	2.0 g	1.0 g
Nicotine	active drug	<---------- 2.5 mg/cm^2 ---------->				
Dibutyl pthalate	plasticizer	<---------- 5 % of polymer ---------->				
PG	cosolvent	<---------- 10 % of polymer ---------->				
BHA	antioxidant	<---------- 0.1 % ---------->				
Mineral oil	cosolvent	-	-	-	-	1.0 g
Methylene chloride qs. to	solvent	5 ml	10 ml	15 ml	20 ml	10 ml

4. Evaluation of Nicotine TDS Formulations

4.1 Physical Characteristics Evaluation of Nicotine-TDS Formulations

All nicotine preparations exhibited a colorless to pale yellow. To determine the stability of the preparation at accelerated condition they were kept at 45°C for 4 weeks. The results was summarized in Table 19.

Table 19 Stability of nicotine-TDS preparations after keeping at 45°C for 4 weeks.

Formula #	Color change
1	+1
2	+4
3	+2
4	+1
5	+1
6	+1
7	+1
8	+1
9	+1
10	+1
11	+1
12	+1
13	+1
14	+1
15	+1

Remark : (+) showed a degree of intensity of color (n = 3)

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

4.2 *In-vitro* Evaluation of Formulations

In-vitro release and skin permeation experiment which used pig's skin, the result of release and skin permeation data of Nicotinell®-TTS and fourteen formulas are summarized in Appendices xvi-xxx and Tables 20-26. The release and skin permeation profiles of the fourteen formulas compared to Nicotinell®-TTS were showed in Figures 31-52. All data were presented as average cumulative permeation of nicotine through membrane, adhesive and skin. All preparations, exactly, sustained permeation of nicotine over 24 hours.

Table 20 The average cumulative amount of nicotine release per surface area (mg/cm^2) from nicotine-TDS in mineral oil vehicles as compared to Nicotinell® -TTS (n=3).

Time (hrs)	Cumulative released (mg/cm^2) ± SD		
	Nicotinell®-TTS	Formula #1	Formula #3
0	0	0	0
0.5	0.4145 ± 0.0080	0.6031 ± 0.0386	0.1364 ± 0.0080
1	0.5898 ± 0.0090	0.7742 ± 0.0443	0.2655 ± 0.0245
2	0.7822 ± 0.0169	1.0304 ± 0.0641	0.3918 ± 0.0328
3	0.9549 ± 0.0031	1.1572 ± 0.0702	0.4965 ± 0.0195
4	1.0727 ± 0.0094	1.2912 ± 0.1151	0.6834 ± 0.0139
6	1.3195 ± 0.0086	1.4822 ± 0.1131	0.8615 ± 0.0130
8	1.4249 ± 0.0059	1.6459 ± 0.1565	1.0603 ± 0.0889
10	1.5200 ± 0.0290	1.7592 ± 0.1334	1.2559 ± 0.0819
12	1.5910 ± 0.0051	1.8578 ± 0.1360	1.4759 ± 0.1059
14	1.6540 ± 0.0111	1.988 ± 0.1546	1.5856 ± 0.1067
16	1.7550 ± 0.0081	2.0385 ± 0.1434	1.6755 ± 0.1001
20	1.8359 ± 0.0041	2.1011 ± 0.1511	1.8281 ± 0.0995
24	1.9303 ± 0.0009	2.3153 ± 0.1953	2.0195 ± 0.1036

Table 21 The average cumulative amount of nicotine release per surface area (mg/cm^2) from nicotine-TDS in carbomer 934 systems (n=3).

Time (hrs)	Cumulative released (mg/cm^2) ± SD			
	Formula #4	Formula #5	Formula #6	Formula #7
0	0	0	0	0
0.5	0.4079 ± 0.0087	0.2788 ± 0.0222	0.1055 ± 0.0048	0.1798 ± 0.0095
1	0.5289 ± 0.0215	0.4089 ± 0.0089	0.1701 ± 0.0125	0.2549 ± 0.0080
2	0.6828 ± 0.0157	0.5431 ± 0.0055	0.2678 ± 0.0078	0.3495 ± 0.0179
3	0.7675 ± 0.0117	0.7106 ± 0.0281	0.3282 ± 0.0099	0.4045 ± 0.0258
4	0.8445 ± 0.0176	0.7863 ± 0.0071	0.3805 ± 0.0102	0.4901 ± 0.0170
6	1.0349 ± 0.0163	0.9302 ± 0.0156	0.5318 ± 0.0227	0.6481 ± 0.0146
8	1.1881 ± 0.0142	1.0330 ± 0.0353	0.6819 ± 0.0426	0.7389 ± 0.0104
10	1.3021 ± 0.0277	1.2056 ± 0.0124	0.8684 ± 0.0503	0.8315 ± 0.0126
12	1.4618 ± 0.0097	1.3322 ± 0.0234	0.9298 ± 0.0358	0.8839 ± 0.0114
14	1.5564 ± 0.0114	1.5171 ± .0469	1.0582 ± 0.0558	0.9670 ± 0.0256
16	1.6524 ± 0.0315	1.6691 ± 0.0551	1.1317 ± 0.0646	1.0354 ± 0.0294
20	1.8003 ± 0.0438	1.8129 ± 0.0527	1.3378 ± 0.0709	1.1386 ± 0.0298
24	1.9672 ± 0.0219	1.9768 ± 0.0521	1.5027 ± 0.0640	1.2248 ± 0.0426

Table 22 Average cumulative amount of nicotine release per surface area (mg/cm^2) from nicotine-TDS in pluronic F-127 systems (n=3).

Time (hrs)	Cumulative released (mg/cm^2) ± SD		
	Formula #8	Formula #9	Formula #10
0	0	0	0
0.5	0.1569 ± 0.0050	0.0858 ± 0.0142	-
1	0.1989 ± 0.0080	0.1388 ± 0.0114	0.0378 ± 0.0035
2	0.2905 ± 0.0100	0.2129 ± 0.0195	0.0846 ± 0.0043
3	0.3389 ± 0.0148	0.2737 ± 0.0241	0.1382 ± 0.0062
4	0.3831 ± 0.0100	0.3419 ± 0.0452	0.1863 ± 0.0016
6	0.4745 ± 0.0209	0.4886 ± 0.0289	0.2463 ± 0.0186
8	0.5579 ± 0.0353	0.5432 ± 0.0039	0.3550 ± 0.0216
10	0.6380 ± 0.0152	0.6598 ± 0.0334	0.4698 ± 0.0236
12	0.7196 ± 0.0223	0.7234 ± 0.0145	0.5003 ± 0.0428
14	0.7569 ± 0.0207	0.7970 ± 0.0259	0.5855 ± 0.0159
16	0.7852 ± 0.0423	0.8737 ± 0.0204	0.6224 ± 0.0108
20	0.9337 ± 0.0318	0.9755 ± 0.0418	0.7048 ± 0.0065
24	1.0186 ± 0.0272	1.0512 ± 0.0398	0.7527 ± 0.0159

Table 23 Average cumulative amount of nicotine release per surface area (mg/cm^2) from nicotine-TDS in EVA copolymer systems (n=3).

Time	Cumulative released (mg/cm2) ± SD				
(hrs)	Formula #11	Formula #12	Formula #13	Formula #14	Formula #15
0	0	0	0	0	0
0.5	0.4973 ± 0.0176	0.2913 ± 0.0198	0.1354 ± 0.0064	0.2178 ± 0.0227	0.3132 ± 0.0230
1	0.6339 ± 0.0256	0.4044 ± 0.0257	0.1745 ± 0.0109	0.3137 ± 0.0153	0.6134 ± 0.0307
2	0.8559 ± 0.0064	0.4758 ± 0.0123	0.2322 ± 0.0124	0.3687 ± .0134	0.7829 ± 0.0532
3	0.9877 ± 0.0278	0.5809 ± 0.0210	0.3257 ± 0.0117	0.4279 ± 0.0104	0.9902 ± 0.0293
4	1.0872 ± 0.0250	0.6376 ± 0.0283	0.3925 ± 0.0103	0.4617 ± 0.0047	1.1428 ± 0.0128
6	1.2350 ± 0.0069	0.6756 ± 0.0095	0.4389 ± 0.0142	0.4989 ± 0.0019	1.4176 ± 0.0154
8	1.3239 ± 0.0120	0.7729 ± 0.0207	0.5398 ± 0.0114	0.5469 ± 0.0078	1.5006 ± 0.0131
10	1.3904 ± 0.0210	0.8422 ± 0.0094	0.5834 ± 0.0119	0.5867 ± 0.0089	1.5725 ± 0.0047
12	1.4817 ± 0.0225	0.9190 ± 0.0227	0.6469 ± 0.0121	06515 ± 0.0082	1.7603 ± 0.0223
14	1.5471 ± 0.0190	1.0022 ± 0.0127	0.7569 ± 0.0226	0.6892 ± 0.0098	1.9060 ± 0.0093
16	1.7124 ± 0.0090	1.0912 ± 0.0162	0.8479 ± 0.0181	0.7377 ± 0.0120	1.9809 ± 0.0130
20	1.7705 ± 0.0145	1.1944 ± 0.0117	0.9789 ± 0.0280	0.8429 ± 0.0129	2.0307 ± 0.0119
24	1.8139 ± 0.0123	1.3323 ± 0.0137	1.1500 ± 0.0200	0.8976 ± 0.0169	2.1236 ± 0.0109

Table 24 Average cumulative amount of nicotine skin permeation per surface area (mg/cm^2) from nicotine-TDS in mineral oil vehicle as compared to Nicotinell®-TTS (n=3).

Time	Cumulative skin permeation (mg/cm^2) ± SD		
(hrs)	Nicotinell®-TTS	Formula #1	Formula #3
0	0	0	0
0.5	0.0826 ± 0.0019	0.0793 ± 0.0015	0.1044 ± 0.0019
1	0.1591 ± 0.0027	0.1559 ± 0.0071	0.2130 ± 0.0149
2	0.3201 ± 0.0072	0.2939 ± 0.0132	0.3557 ± 0.0089
3	0.4448 ± 0.0047	0.4308 ± 0.0211	0.4878 ± 0.0209
4	0.5352 ± 0.0167	0.5272 ± 0.0181	0.5878 ± 0.0193
6	0.7653 ± 0.0188	0.6957 ± 0.0319	0.7658 ± 0.0216
8	0.9566 ± 0.0176	0.8624 ± 0.0164	0.9524 ± 0.0368
10	1.0957 ± 0.0131	1.0391 ± 0.0145	1.1286 ± 0.0330
12	1.2636 ± 0.0276	1.1717 ± 0.0177	1.3239 ± 0.0183
14	1.4074 ± 0.0370	1.3072 ± 0.0155	1.4748 ± 0.0329
16	1.564 ± 0.0423	1.5014 ± 0.0199	1.5788 ± 0.0176
24	1.8449 ± 0.0381	1.8991 ± 0.0238	1.8243 ± 0.0186

Table 25 Average cumulative amount of nicotine skin permeation per surface area (mg/cm²) from nicotine-TDS in carbomer 934 systems (n=3).

Time (hrs)	Cumulative skin permeation (mg/cm2) ± SD			
	Formula #4	Formula #5	Formula #6	Formula #7
0	0	0	0	0
0.5	0.1268 ± 0.0165	0.1344 ± 0.0295	0.0292 ± 0.0018	0.0253 ± 0.0017
1	0.2528 ± 0.0118	0.2076 ± 0.0059	0.0656 ± 0.0043	0.0515 ± 0.0058
2	0.4294 ± 0.0248	0.3556 ± 0.0545	0.1508 ± 0.0071	0.1039 ± 0.0068
3	0.5594 ± 0.0256	0.4358 ± 0.0312	0.2262 ± 0.0129	0.1605 ± 0.0187
4	0.6742 ± 0.0187	0.5253 ± 0.0223	0.2969 ± 0.0157	0.2147 ± 0.0079
6	0.8841 ± 0.0157	0.6519 ± 0.0114	0.4338 ± 0.0286	0.3283 ± 0.0061
8	1.0614 ± 0.0203	0.7743 ± 0.0305	0.5447 ± 0.0307	0.4238 ± 0.0052
10	1.2056 ± 0.0451	0.9352 ± 0.0231	0.6594 ± 0.0339	0.4993 ± 0.0080
12	1.3219 ± 0.0322	1.0911 ± 0.0395	0.7568 ± 0.0414	0.5929 ± 0.015
14	1.4772 ± 0.0554	1.1939 ± 0.0465	0.8326 ± 0.0455	0.6552 ± 0.0063
16	1.6085 ± 0.0792	1.3719 ± 0.0767	0.9733 ± 0.0532	0.7071 ± 0.0308
20	1.7844 ± 0.0643	1.5168 ± 0.0355	1.0489 ± 0.0591	0.8168 ± 0.0181
24	1.9094 ± 0.0277	1.6760 ± 0.0559	1.1325 ± 0.0618	0.9149 ± 0.0027

Table 26 Average cumulative amount of nicotine skin permeation per surface area (mg/cm²) from nicotine-TDS in EVA copolymer systems (n=3).

Time (hrs)	Cumulative skin permeation (mg/cm²) ± SD				
	Formula #11	Formula #12	Formula #13	Formula #14	Formula #15
0	0	0	0	0	0
0.5	0.0615 ± 0.0429	0.0404 ± 0.0036	0.0595 ± 0.0196	0.0472 ± 0.0046	0.0619 ± 0.0045
1	0.3105 ± 0.0206	0.4730 ± 0.0078	0.0802 ± 0.0013	0.0796 ± 0.0051	0.1360 ± 0.0045
2	0.4629 ± 0.0071	0.1589 ± 0.0068	0.1117 ± 0.0008	0.1249 ± 0.0069	0.2733 ± 0.0162
3	0.6065 ± 0.0182	0.2553 ± 0.0122	0.2009 ± 0.0062	0.1694 ± 0.0007	0.4416 ± 0.0033
4	0.6919 ± 0.0343	0.3128 ± 0.0183	0.2906 ± 0.0084	0.2109 ± 0.0054	0.5300 ± 0.0127
6	0.7789 ± 0.0210	0.4618 ± 0.0107	0.4613 ± 0.0052	0.4318 ± 0.0226	0.7003 ± 0.0128
8	1.0525 ± 0.0397	0.5581 ± 0.0189	0.5629 ± 0.0075	0.5226 ± 0.0214	0.9485 ± 0.0009
10	1.3084 ± 0.0277	0.6612 ± 0.0207	0.6401 ± 0.0089	0.6002 ± 0.0130	1.0826 ± 0.0422
12	1.4443 ± 0.0152	0.7626 ±0.0101	0.7329 ± 0.0197	0.6605 ± 0.0106	1.3099 ± 0.0139
14	1.5579 ± 0.0154	0.9012 ± 0.0134	0.8100 ± 0.0169	0.7183 ± 0.0097	1.4537 ± 0.0421
16	1.7264 ± 0.0363	0.9424 ± 0.0096	0.8979 ± 0.0102	0.8247 ± 0.0255	1.5354 ± 0.0421
20	1.8073 ± 0.0954	1.1138 ± 0.0168	0.9919 ± 0.0115	0.9588 ± 0.0134	1.6709 ± 0.0176
24	1.9363 ± 0.0259	1.2222 ± 0.0188	1.1435 ± 0.0118	1.0782 ± 0.0459	1.8443 ± 0.0213

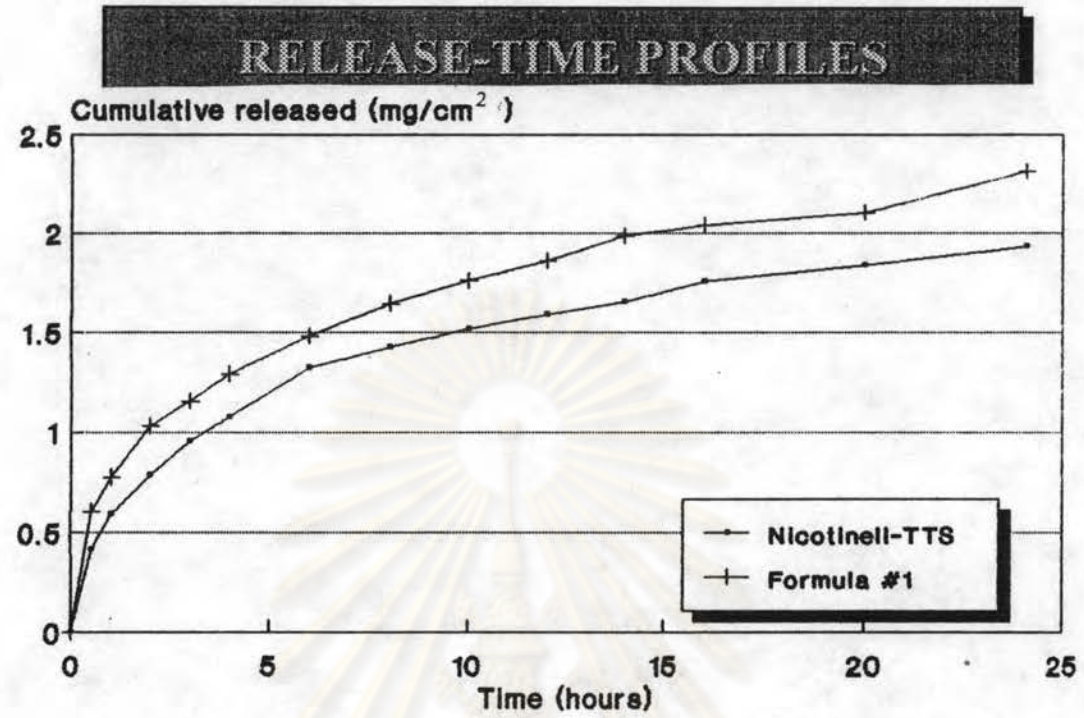


Figure 32 Average release-time profiles of Formula #1compared to Nicotinell®-TTS (n=3).

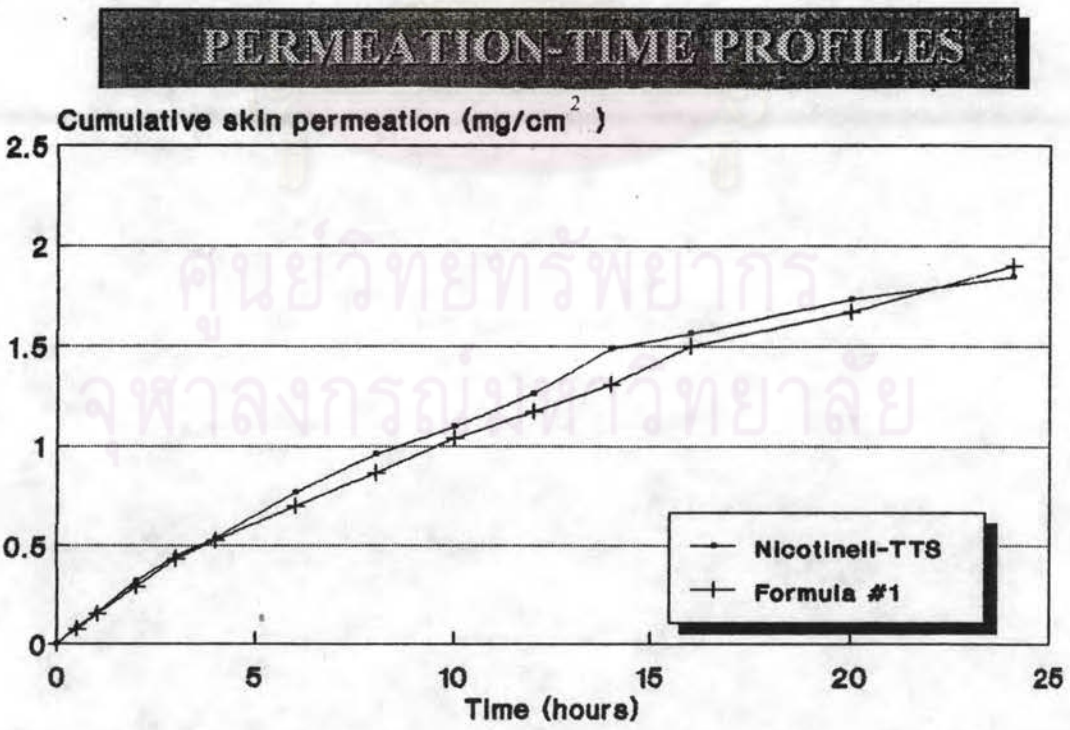


Figure 33 Average skin permeation-time profiles of Formula #1 compared to Nicotinell®-TTS (n=3).

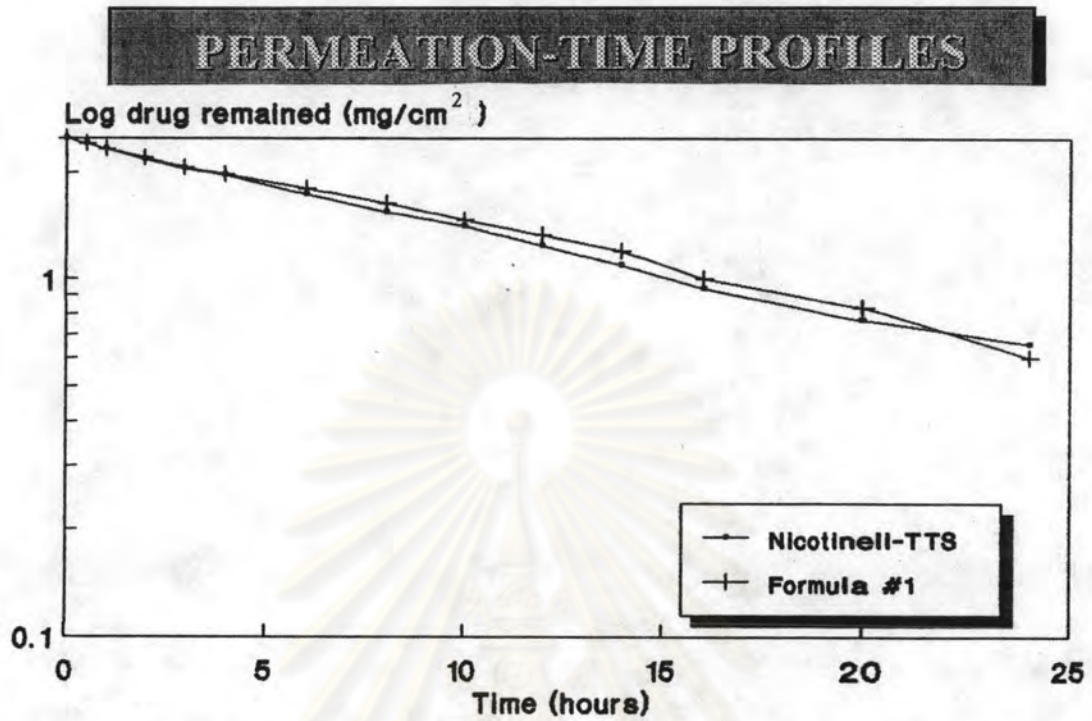


Figure 34 Average logarithm of drug remaining-time profiles of Formula #1 compared to Nicotinell®-TTS (n=3).

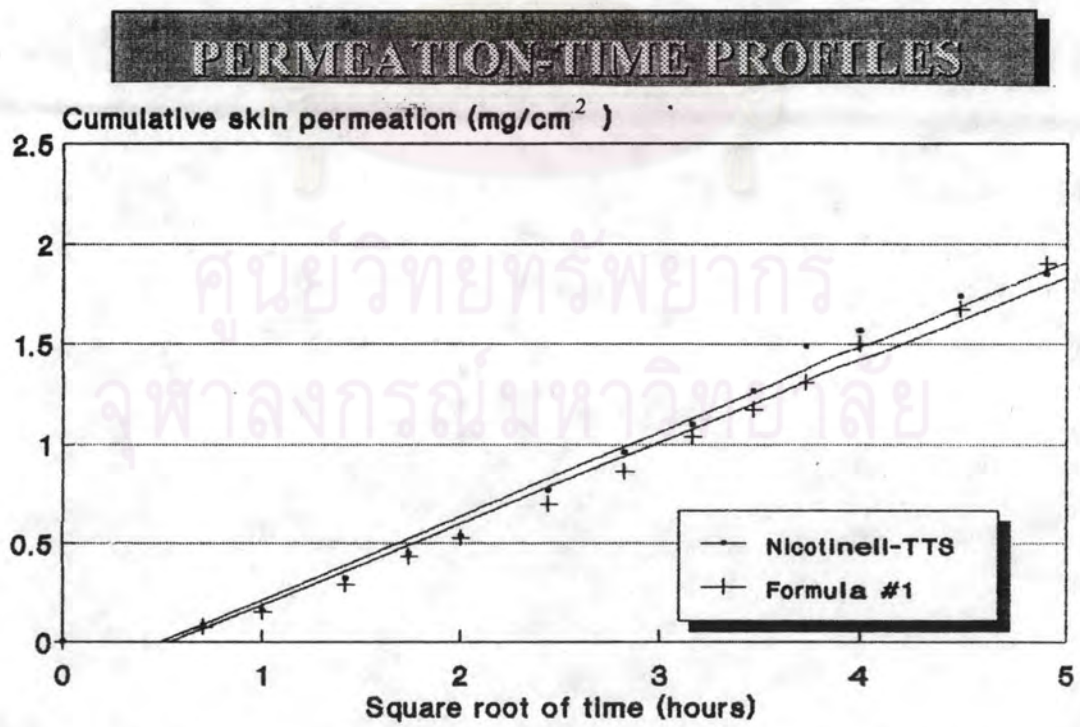


Figure 35 Average skin permeation-square root of time profiles of Fomula#1 compared to Nicotinell®-TTS (n=3).

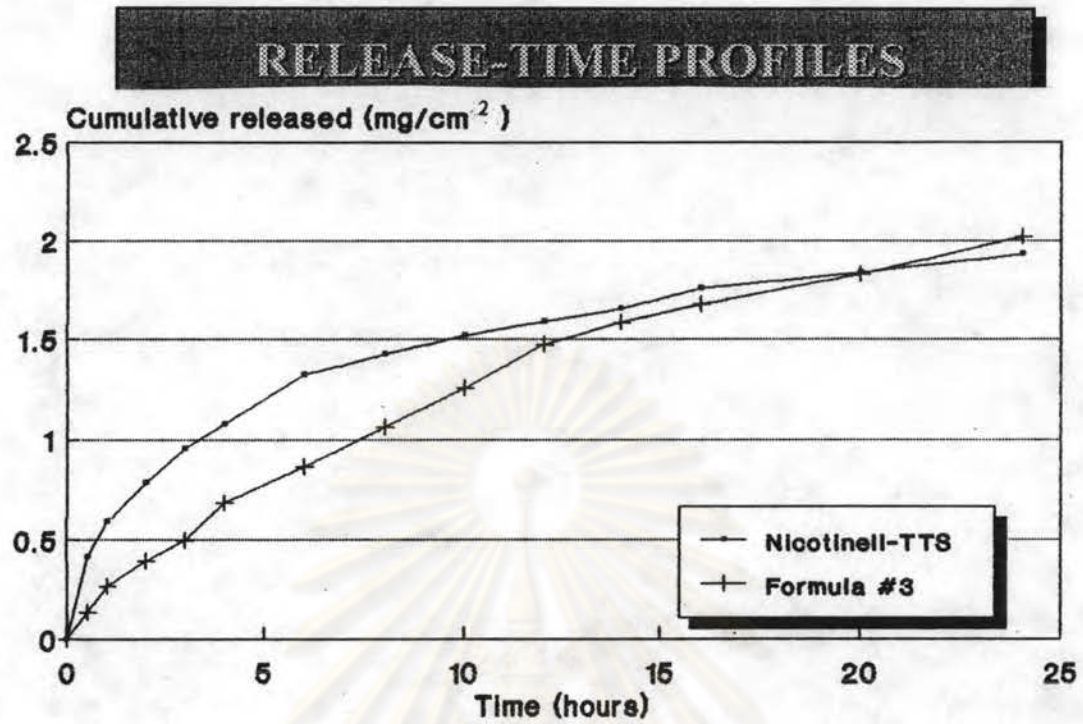


Figure 36 Average release-time profiles of Formula #3 compared to Nicotinell®-TTS (n=3).

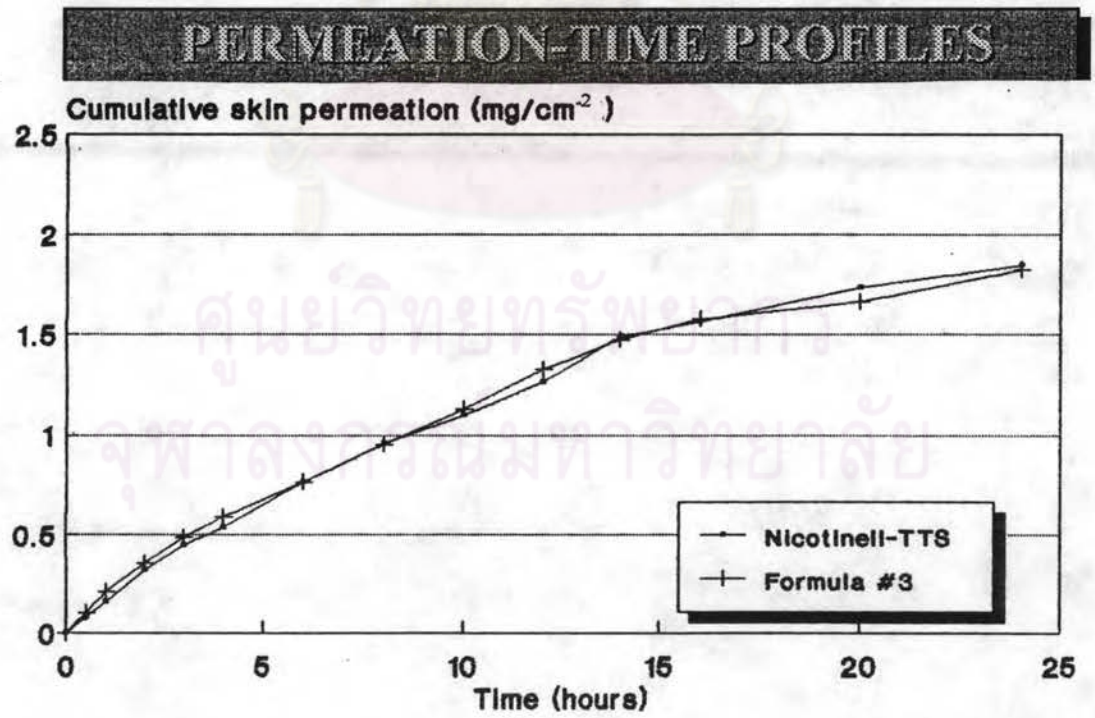


Figure 37 Average skin permeation-time profiles of Formula #3 compared to Nicotinell®-TTS (n=3).

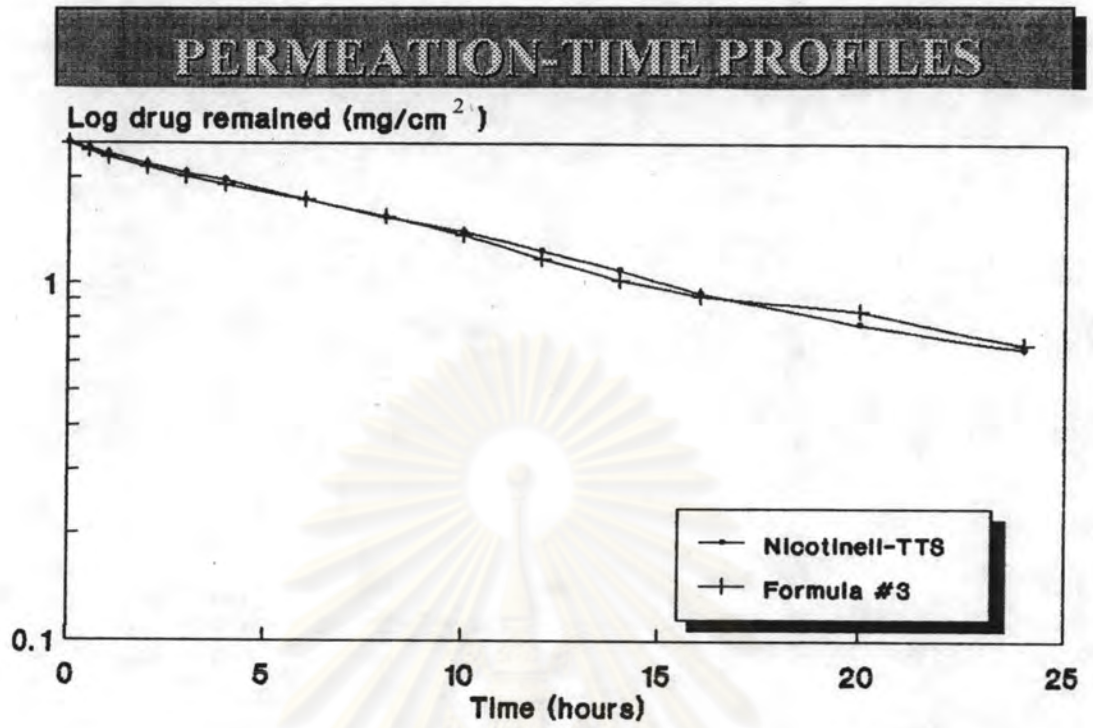


Figure 38 Average logarithm of drug remaining-time profiles of Formula #3 compared to Nicotinell®-TTS (n=3).

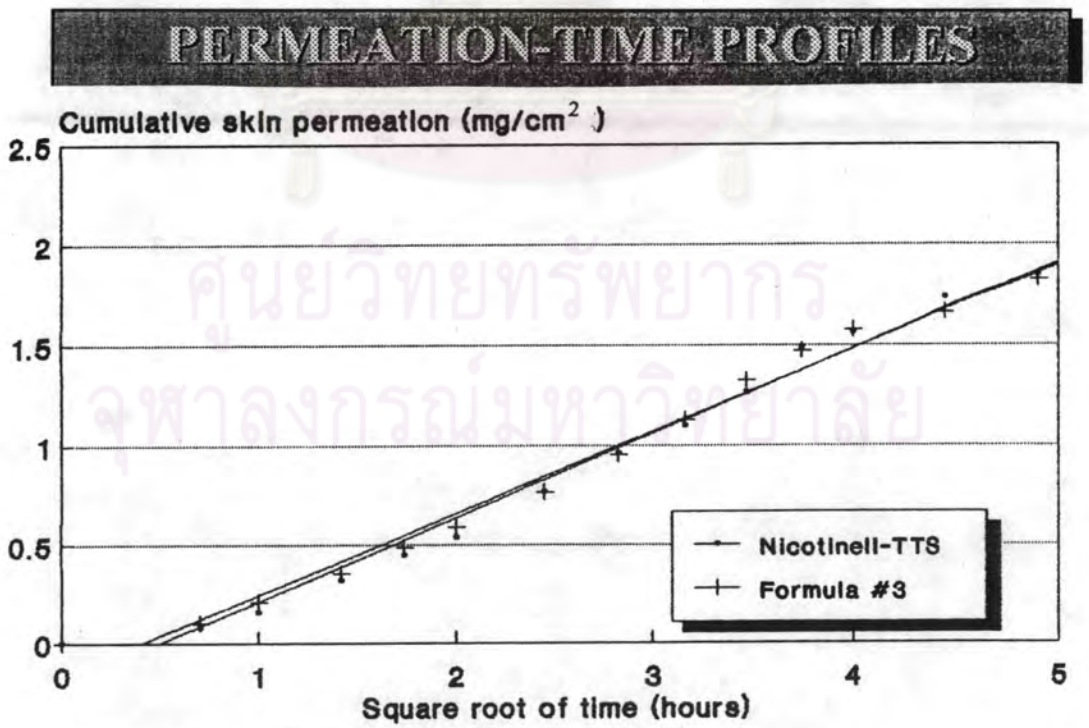


Figure 39 Average skin permeation-square root of time profiles of Formula #3 compared to Nicotinell®-TTS (n=3).

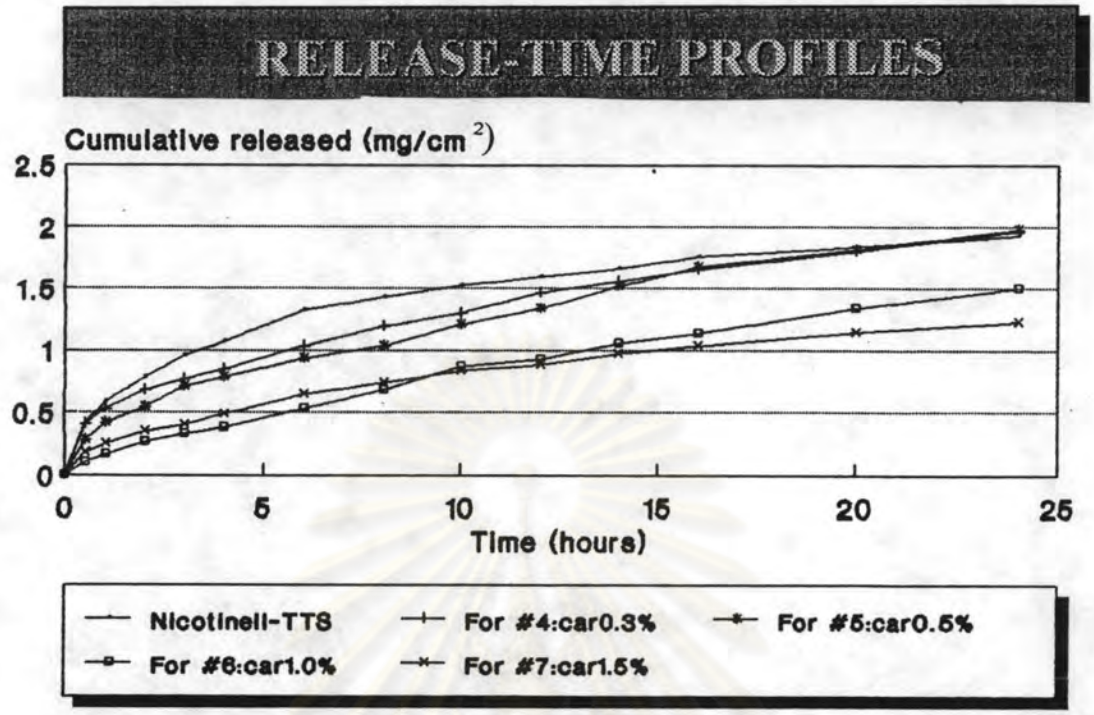


Figure 40 Average release-time profiles of Formula # 4-7 compared to Nicotinell®-TTS (n=3).

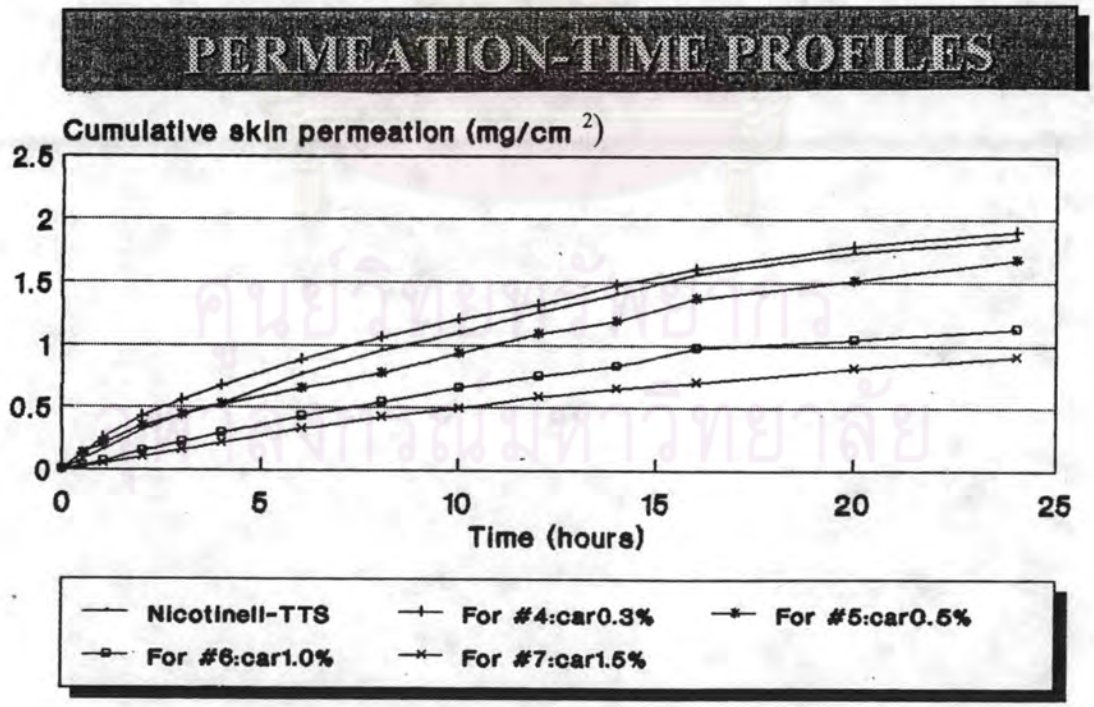


Figure 41 Average skin permeation-time profiles of Formula # 4-7 compared to Nicotinell®-TTS (n=3).

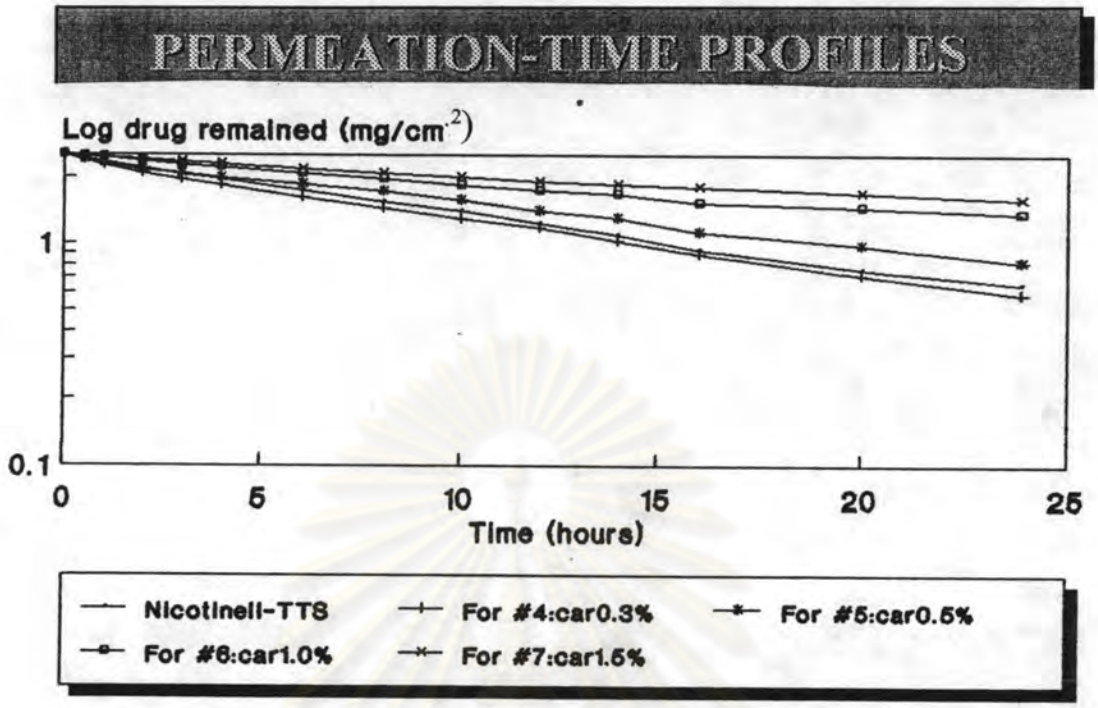


Figure 42 Average logarithm of drug remaining-time profiles of Formula #4-7 compared to Nicotinell®-TTS (n=3).

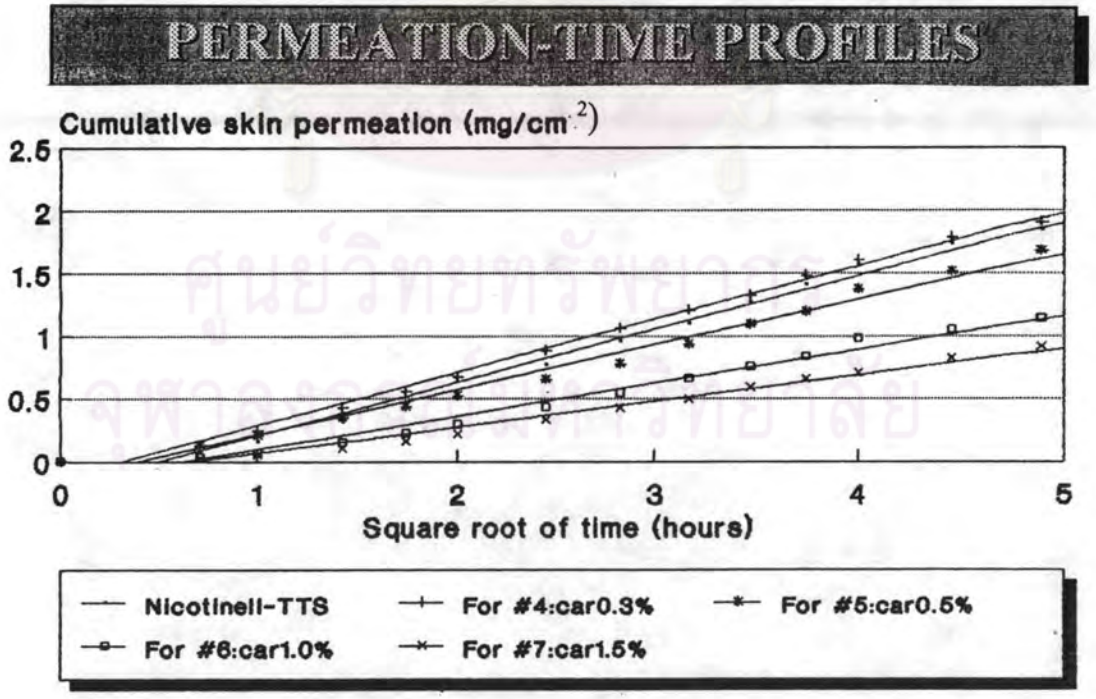


Figure 43 Average skin permeation-square root of time profiles of Formula #4-7 compared to Nicotinell®-TTS (n=3).

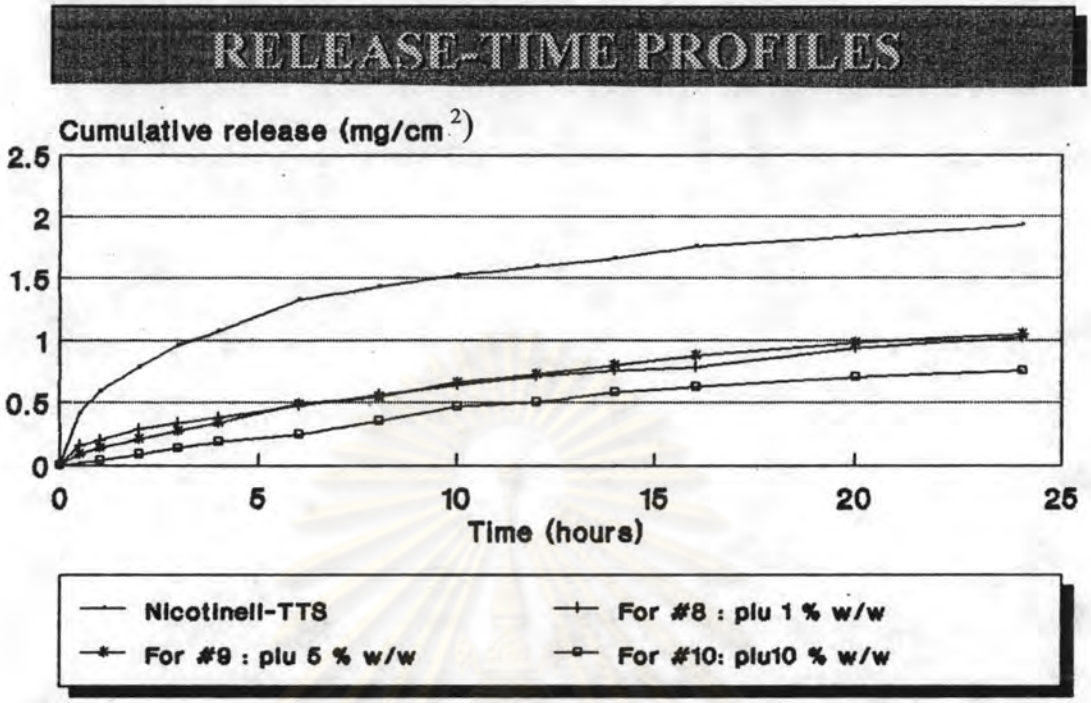


Figure 44 Average release-time profiles of Formula #8-10 compared to Nicotinell®-TTS (n=3).

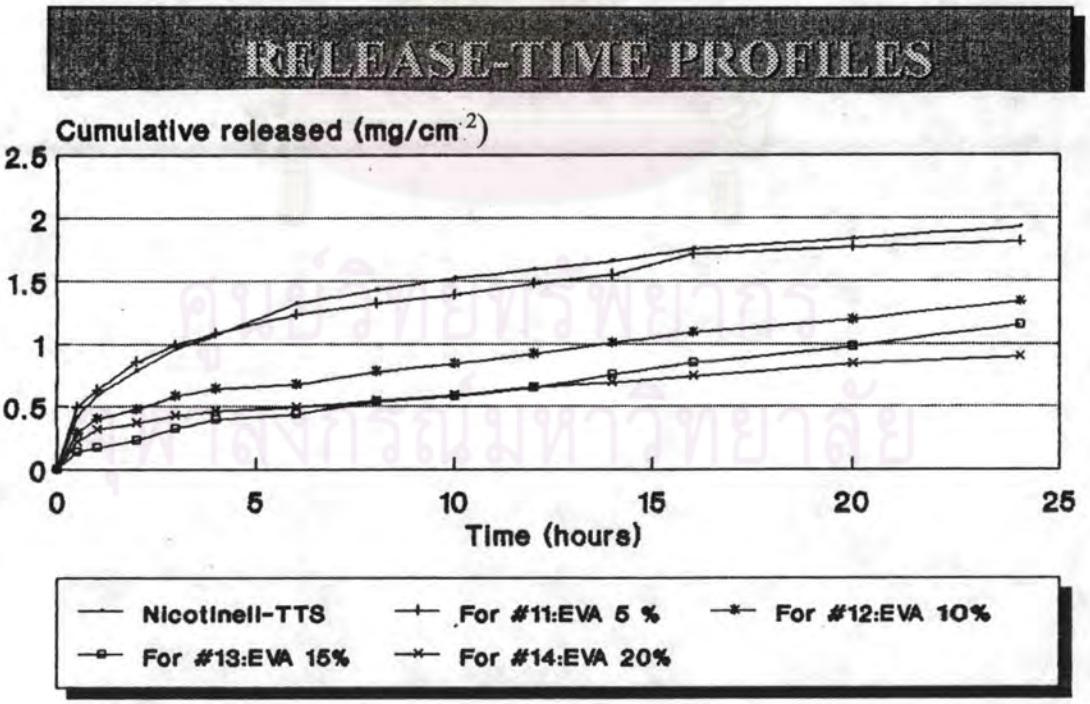


Figure 45 Average release-time profiles of Formula #11-14 compared to Nicotinell®-TDS (n=3) .

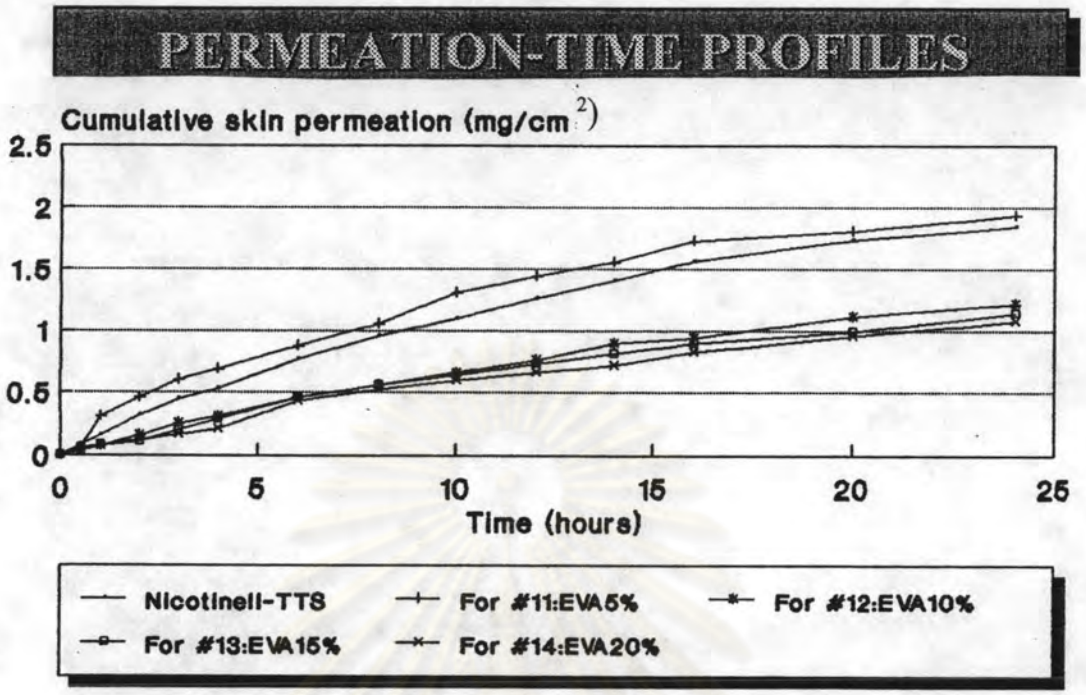


Figure 46 Average skin permeation-time profiles of Formula #11-14 compared to Nicotinell®-TTS (n=3).

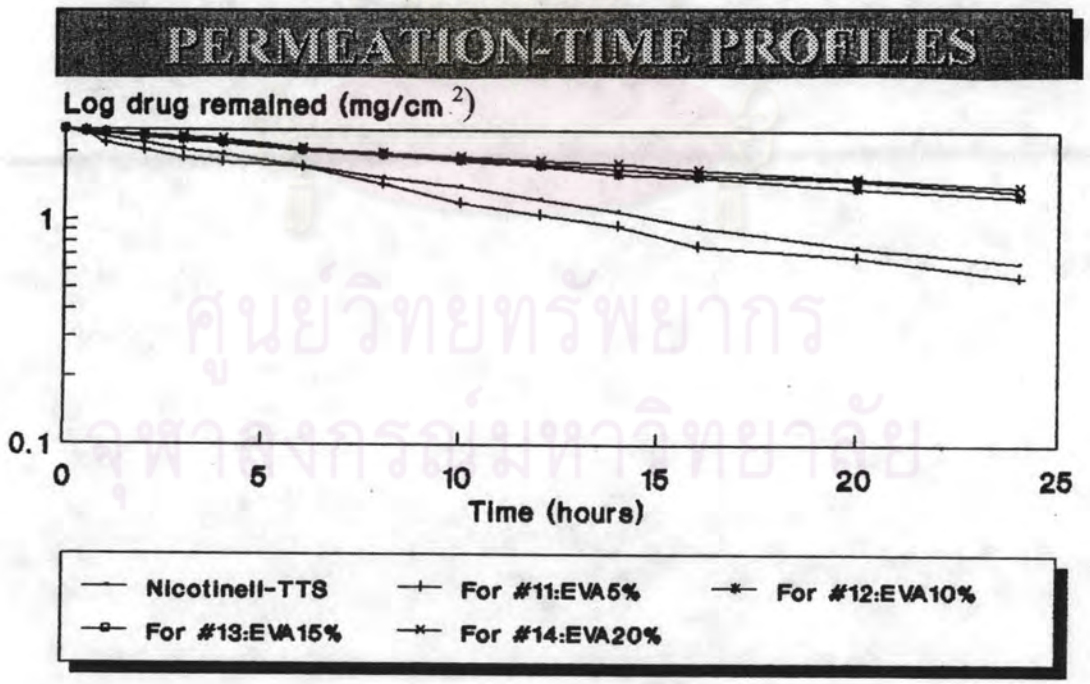


Figure 47 Average logarithm of drug remained-time profiles of Formula #11-14 compared to Nicotinell®-TTS (n=3).

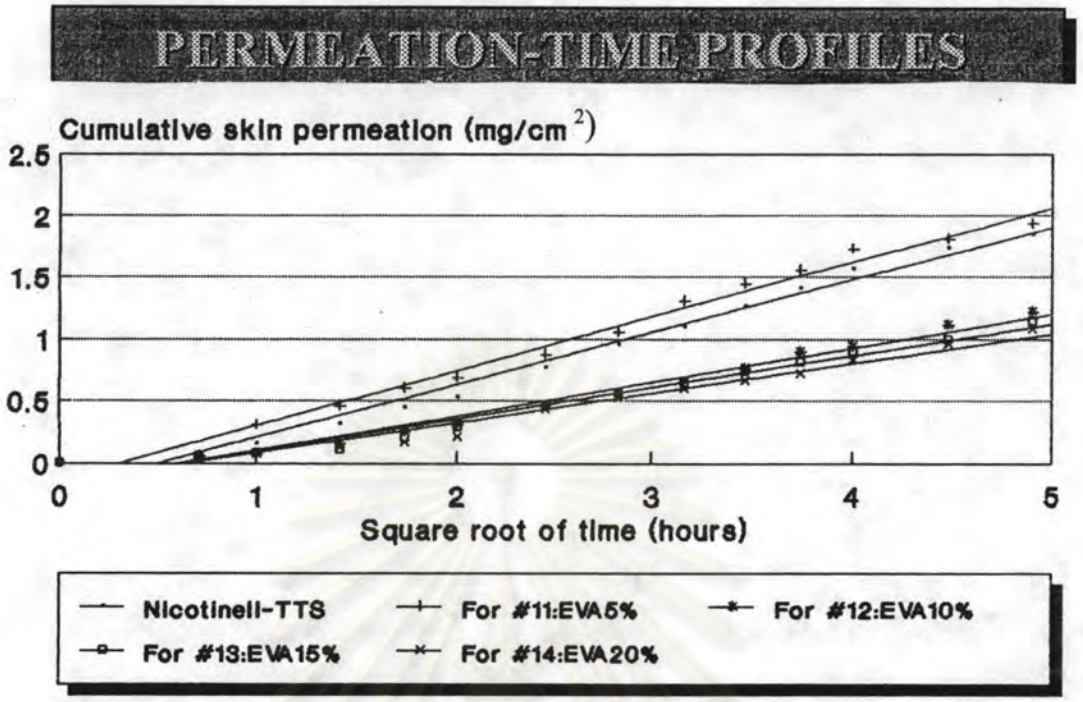


Figure 48 Average skin permeation-square root of time profiles of Formula #11-14 compared toNicotinell®-TTS (n=3).

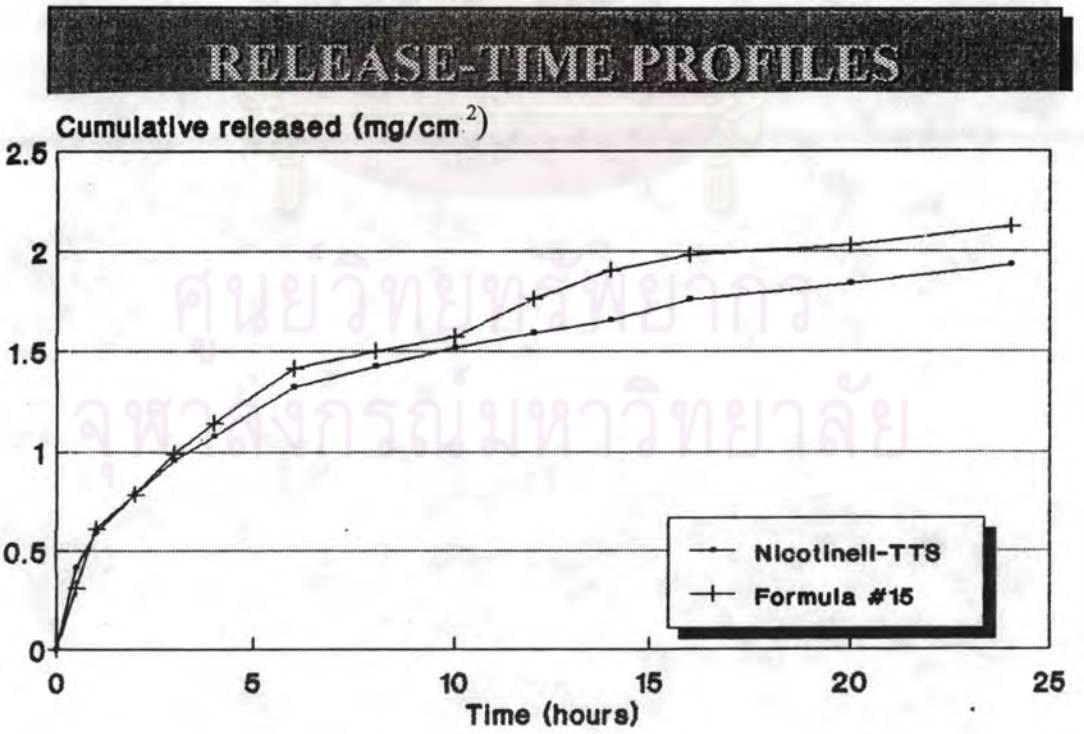


Figure 49 Average release-time profiles of Formula #15 compared to Nicotinell®-TDS (n=3).

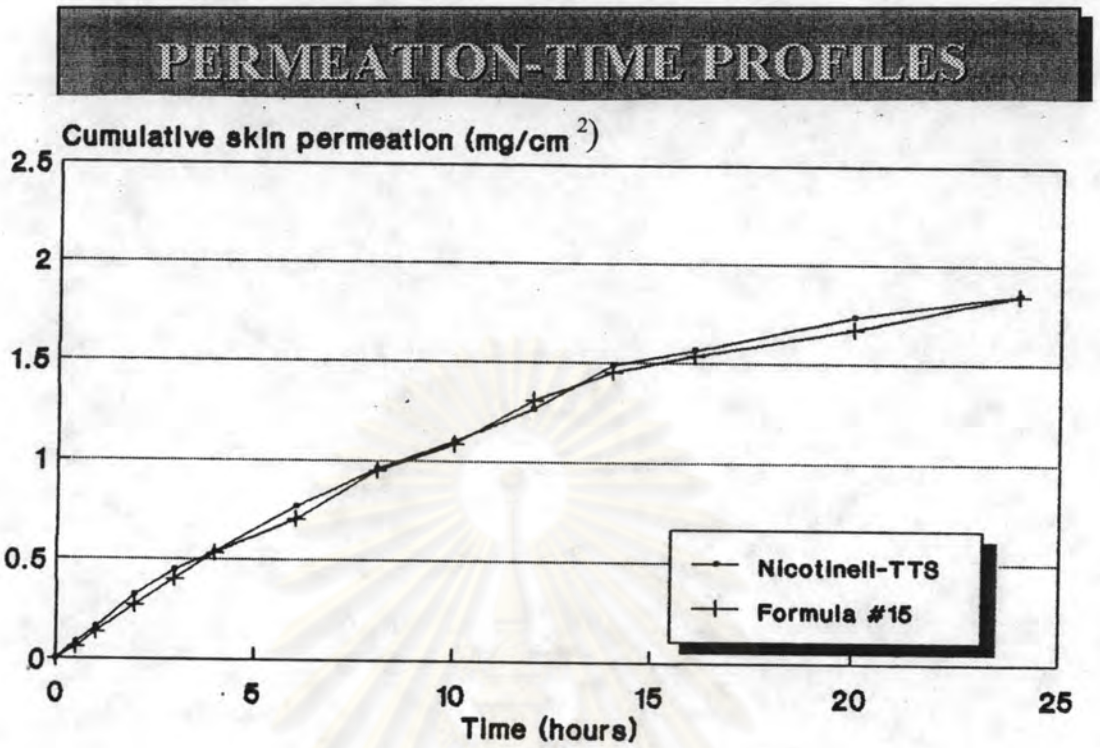


Figure 50 Average skin permeation-time profiles of Formula # 15 compared to Nicotinell®-TTS (n=3).

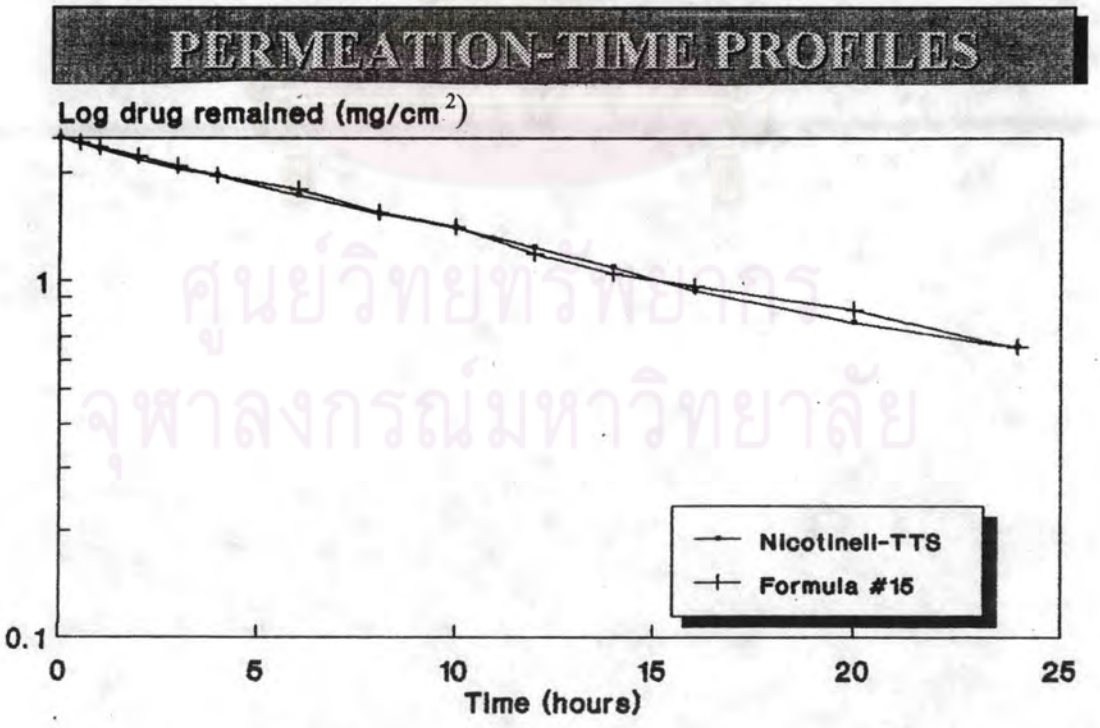


Figure 51 Average logarithm of drug remaining-time profiles of Formula # 15 compared to Nicotinell®-TTS (n=3).

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

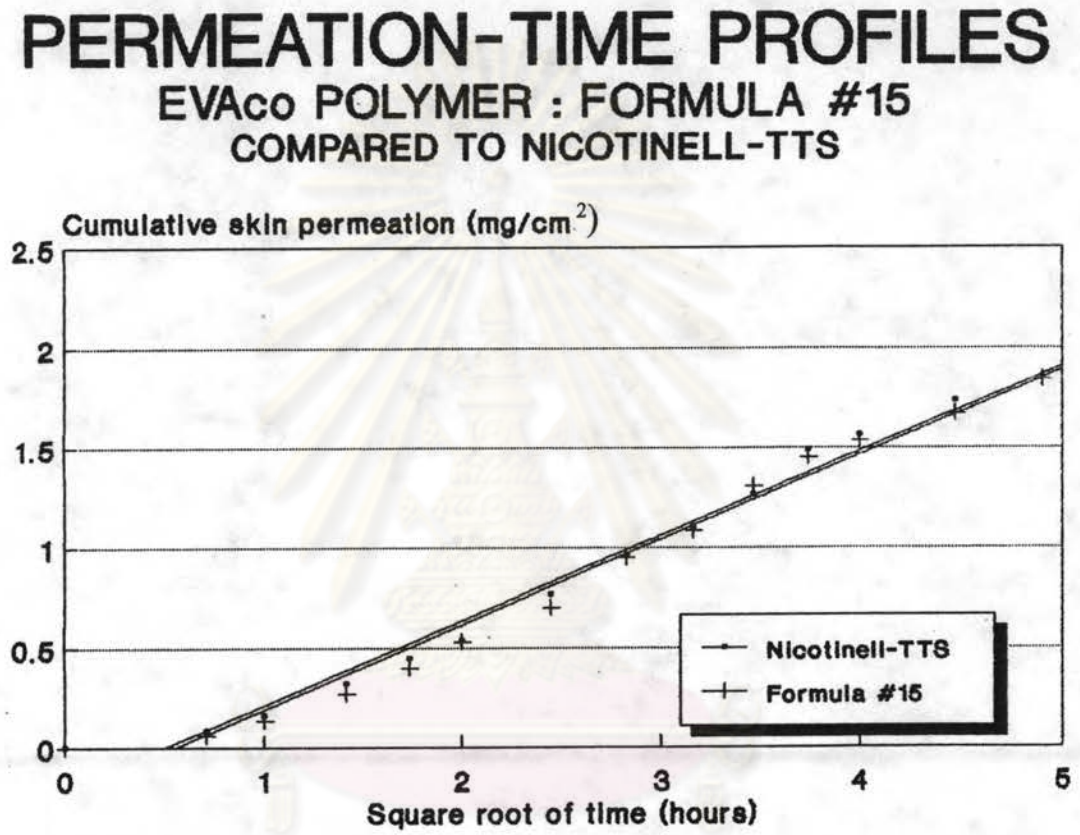


Figure 52 Average skin permeation-square root of time profiles of Formula #15 compared to Nicotinell®-TTS (n=3).

In order to determine the effects of different type of gelling agent and different formulation on the model of drug permeation, all of the release and skin permeation data were fitted to zero order, first order or Higuchi's model. The most linearity which obtained form correlate between Q_s versus time (zero order kinetic), the logarithm of remained Q_s versus time (first order kinetic) and Q_s versus square root of time (Higuchi's model) were used to determine the kinetics of drug release and permeation model of each formulations (Tables 27-28).

The release and skin permeation mechanism is based on equation:

$$M_t/M_\alpha = Kt^n \quad \text{.....(20)}$$

Where M_t/M_α is the fraction of drug release up to time t, K is a proportionallity constant incorporating structural and geometric characteristics of the controlled device, n is the diffusion release

The relationship between the diffusional exponent n and the corresponding release mechanism is shown in Appendix xxxi. The values of n were analyzed by Data Test Computer Program developed by Kulvanich, p., et al. as showed in Tables 27-28. The value of n is 0.5 for Fickian diffusion, when the value of n is > 0.5 and < 1.0, the release was said to be non Fickian. A value of $n = 1$ mean the drug is zero order release.

From drug release mechanism test, n release exponent of skin permeation were higher than release without skin. The release exponent n of Nicotinell®-TTS was 0.75 and the release exponents n of the prepared formulas were nearly commercial products (Nicotinell®-TTS). These indicated that the release mechanism through skin of all formulations were anomalous transport.

Table 27 The analysis of nicotine release kinetics and mechanism in various formulas.

Formula #	Correlation coefficient			Kinetic	$M_t/M_\infty = Kf^n$			Mechanism
	Q vs t	logrQ vs t	Q vs sqr of t	pattern	n	K	r^2	pattern
Nicotinell®	0.8589	0.955	0.9684	Higuchi	0.44	0.233271	0.996644	non-Fickian
1	0.8817	0.9688	0.991	Higuchi	0.36	0.31296	0.99829	non-Fickian
3	0.9486	0.9971	0.9943	First	0.73	0.094559	0.997489	non-Fickian
4	0.9595	0.9983	0.9984	First	0.42	0.199399	0.99215	non-Fickian
5	0.9689	0.9938	0.993	First	0.47	0.167718	0.995614	non-Fickian
6	0.9859	0.9981	0.9868	First	0.73	0.060584	0.996336	non-Fickian
7	0.9448	0.975	0.997	Higuchi	0.5	0.102167	0.996937	Fickian
8	0.947	0.7433	0.9961	Higuchi	0.52	0.077157	0.993795	non-Fickian
9	0.9598	0.9762	0.9963	Higuchi	0.62	0.060862	0.995309	non-Fickian
10	0.9563	0.9704	0.9888	Higuchi	0.77	0.028197	0.978354	non-Fickian
11	0.8809	0.7736	0.9651	Higuchi	0.33	0.266209	0.991933	non-Fickian
12	0.9329	0.9825	0.9924	Higuchi	0.4	0.142039	0.984777	non-Fickian
13	0.9897	0.9924	0.9966	Higuchi	0.63	0.059036	0.987737	non-Fickian
14	0.9305	0.9549	0.9914	Higuchi	0.36	0.109745	0.98139	non-Fickian
15	0.858	0.969	0.942	First	0.53	0.219816	0.988612	non-Fickian

Table 28 The analysis of nicotine skin permeation kinetics and mechanism in various formulas.

Formula #	Correlation coefficient			Kinetic	$M_t/M_\infty = Kf^n$			Mechanism
	Q vs t	logrQ vs t	Q vs sqr of t	pattern	n	K	r^2	pattern
Nicotinell®	0.9342	0.9969	0.9977	Higuchi	0.75	0.077577	0.997022	non-Fickian
1	0.9808	0.9911	0.9917	Higuchi	0.74	0.073446	0.996311	non-Fickian
3	0.9478	0.9899	0.992	Higuchi	0.64	0.096846	0.992594	non-Fickian
4	0.9508	0.9978	0.9986	Higuchi	0.64	0.10976	0.997599	non-Fickian
5	0.8908	0.9971	0.9989	Higuchi	0.72	0.072598	0.995381	non-Fickian
6	0.9635	0.9843	0.9919	Higuchi	0.75	0.044872	0.98832	non-Fickian
7	0.9732	0.9899	0.9918	Higuchi	0.78	0.031993	0.992369	non-Fickian
11	0.9265	0.9839	0.9868	Higuchi	0.78	0.100793	0.986085	non-Fickian
12	0.9766	0.9165	0.9929	Higuchi	0.76	0.045491	0.994673	non-Fickian
13	0.9667	0.9882	0.9897	Higuchi	0.74	0.044681	0.988156	non-Fickian
14	0.9729	0.9898	0.994	Higuchi	0.78	0.037275	0.98844	non-Fickian
15	0.9534	0.9939	0.9919	First	0.86	0.06261	0.995217	non-Fickian

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย