CHAPTER IV

RESULTS AND DISCUSSION

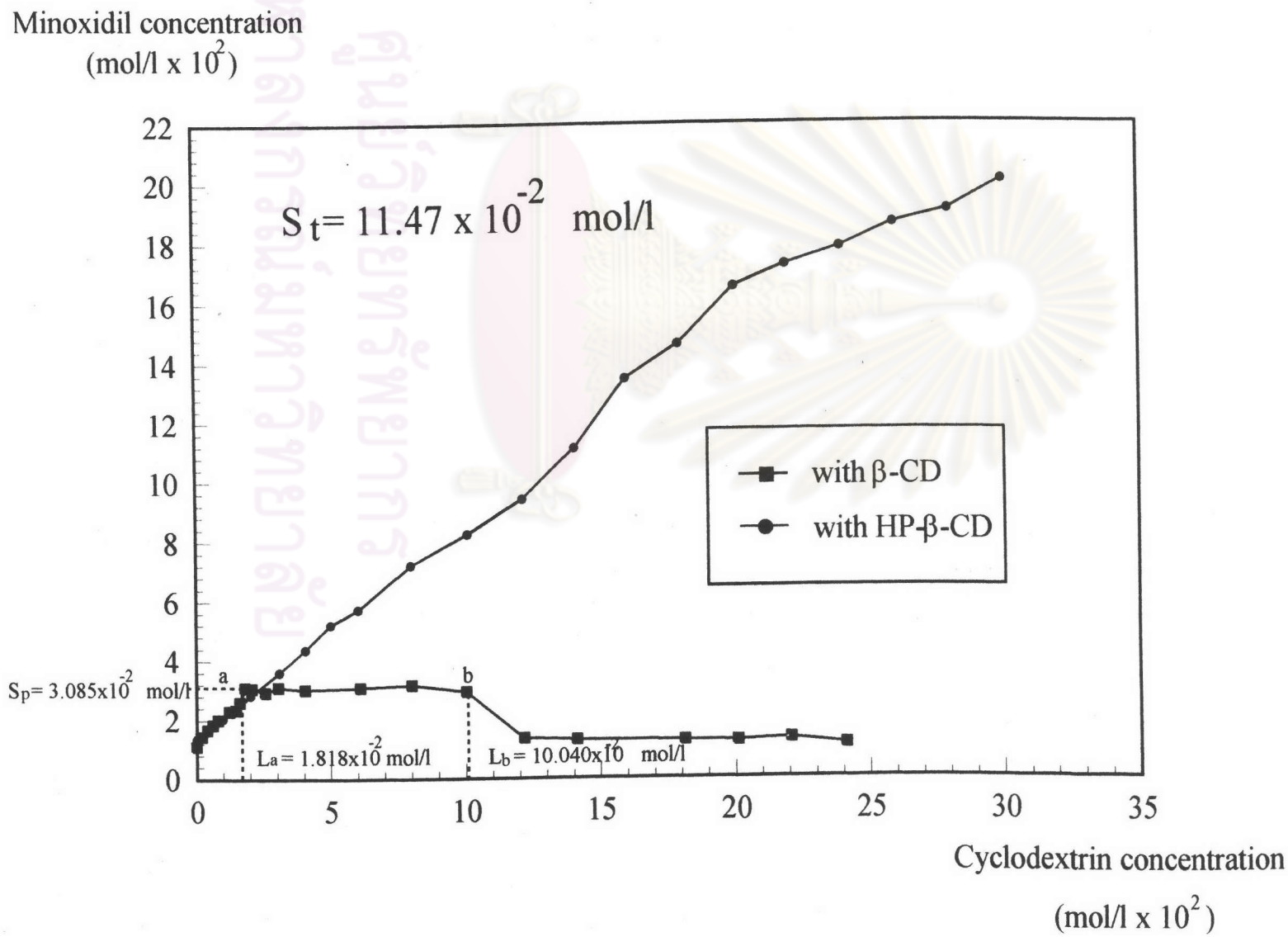
1. Inclusion complexes of minoxidil and CDs in solutions

Phase solubility data between minoxidil and β-CD or HP-β-CD are shown in Appendix I. Phase solubility diagram was constructed by plotting the molarity of minoxidil found in solution against the molarity of CD added. In case of β-CD, the plot showed a B_S type solubility diagram; in contrast, it showed an A_L type in case of HP-β-CD (Figure 7).

In the absence of β-CD, the concentration of minoxidil found in solution was 1.103×10^{-2} mol/l which is the solubility of minoxidil in water at room temperature. As shown in Figure 7, β-CD increased the total solubility of minoxidil to the maximum concentration which reached the solubility limit of the formed complex at about 3.039×10^{-2} mol/l (an average concentration of plateau region "ab" in Figure 7). Thus, the solubility of minoxidil was incerased 2.75 folds over free minoxidil.

On the addition of β-CD, the solubility of minoxidil rose linearly owing to complexation. At point "a", the system was saturated with respect to the complex and to the drug itself. Further addition of β-CD resulted in the continuous formation of the complex which then precipitated out from the saturated solution. At the plateau region, the system still contained excess solid minoxidil, therefore, total solubility of minoxidil in solution remained constant. At point "b", further addition of β-CD into

Figure 7. Phase solubility diagram of minoxidil in water with CDs at 30°C



the system resulted in decreasing water solubility of total minoxidil, since the system no longer had excess solid minoxidil to keep the free minoxidil in solution at the saturated level .

The stoichiometric ratio of the complex could be calculated from the plateau region of the phase solubility diagram. The concentration of β-CD, corresponding to the plateau "ab" equaled to the concentration of β-CD entering the complex over this range. The quantity of minoxidil entering the complex obtained from the undissolved solid remaining at point "a", and could be calculated by subtracting the minoxidil in solution at the saturation point "a" from the total minoxidil initially added. The stoichiometric ratio can be expressed by the following equation (Szejtli, 1982)

$$\text{Stoichiomctric ratio} = \frac{L_b - L_a}{S_t - S_p} \qquad (9)$$

where : L_a, L_b = the amount of ligands in term of molarity at point "a" and "b" on the plateau region, respectively

S_t = total amount of solid substrates in each tube expressed in term of molarity

S_p = the amount of soluble substrates at plateau region

The concentration of β-CD in the plateau region was found to be 10.040×10^{-2} - 1.818×10^{-2} mol/l or 8.222×10^{-2} mol/l. The undissolved solid minoxidil was 11.47×10^{-2} - 3.085×10^{-2} mol/l or 8.385×10^{-2} mol/l, and the stoichiometric ratio was 0.9806. Thus, we can conclude that the stoichiometric ratio of minoxidil : β-CD was 1 : 1.

The formation constant (Kc) of this 1 : 1 complex was calculated from the initial linear portion of the phase solubility diagram according to the following equation (Szejtli, 1982) :

$$Kc = \frac{[\text{minoxidil-}\beta\text{-CD}]}{[\text{minoxidil}]\,[\beta\text{-CD}]} = \frac{\text{slope}}{\text{intercept (1- slope)}} \qquad (10)$$

The slope of the initial straight line and the intercept were 0.9132 (r^2 = 0.9557) and 1.103 x 10^{-2} mol/l, respectively. The formation constant (Kc) was found to be 953.83 l/mol.

In case of minoxidil-HP-β-CD complex, the complex formed was soluble and did not form a precipitate regardless of the amount of HP-β-CD added (Figure 7). Therefore, the phase solubility diagram showed an A_L type with corresponding to 1: 1 complex formation. The formation constant (Kc) was calculated from the phase solubility diagram according to Eq. 10. The slope of the initial straight line and intercept were 0.7386 (r^2 = 0.9981) and 1.359 x 10^{-2} mol/l, respectively. Consequently, the formation constant (Kc) was calculated to be 207.91 l/mol.

From the data obtained, it can be concluded that both β-CD and HP-β-CD increased minoxidil solubility in aqueous solutions.

2. Preparation of minoxidil-CDs solid complexes

The solid complex of minoxidil with β-CD was prepared by stirring the solution containing minoxidil and β-CD at room temperature for 2 days. Since the phase solubility diagram showed the B_S type, the insoluble complex would precipitate out of the solution after an equilibrium was obtained. The solid complex could be isolated by filtration.

On the contrary, the complex of minoxidil with HP-β-CD did not precipitate out of the solution since the phase solubility diagram showed the A_L type. Freeze-drying technique was applied to the complex solution to obtain the solid complex.

3. Investigation of the minoxidil-CDs complexes

3.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry thermograms were shown in Figure 8-9. The endothermic melting point of minoxidil started at 188.2°C and peaked at 193.0°C. In case of β-CD, peak at 95.8°C corresponded to water evaporation and the peak at 217.9°C was the starting degradation peak, but no peak was seen in HP-β-CD.

The thermograms of minoxidil with both CDs showed that the melting point of minoxidil disappeared. On the contrary, their physical mixtures still showed the melting peak of minoxidil, but lower energy was used.

3.2 Infrared spectrophotometry

The IR spectrophotometry study was investigated to verify the complex formation of minoxidil and CDs. The IR spectra of minoxidil, β-CD, HP-β-CD and the complexes were shown in Figure 10-11.

The IR spectra of minoxidil showed major peak at 1640, 1610, 1550, 1231, 1210 and 758 cm^{-1} which corresponded to the following data

N-H	in plane bending	=	1650-1590 cm^{-1}
C=N	stretching	=	1690-1640 cm^{-1}
N-O	stretching	=	1220-1020 cm^{-1}
N-H	out-of-plane bending	=	900-650 cm^{-1}

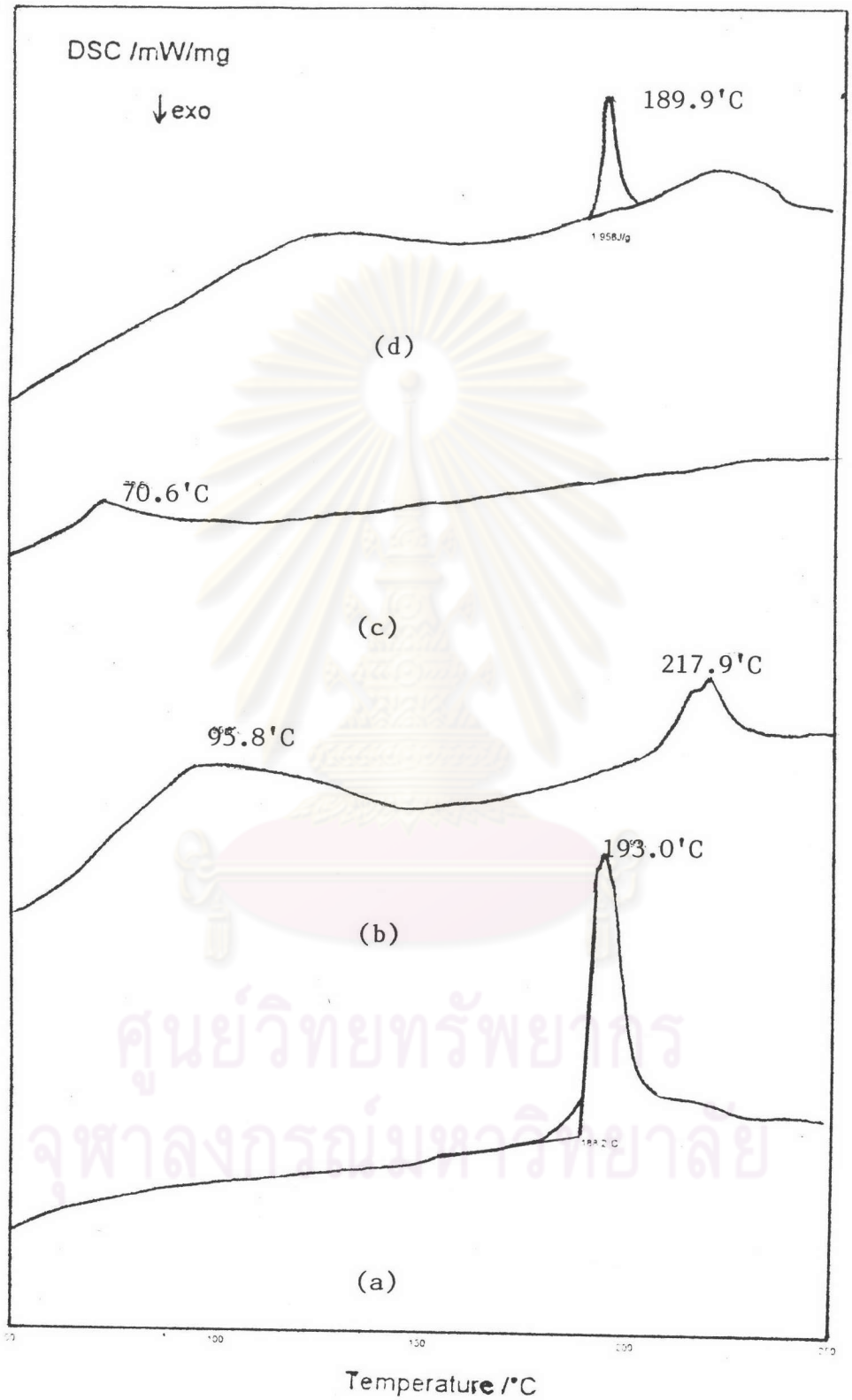


Figure 8. The DSC thermograms of (a) minoxidil; (b) β-CD; (c) minoxidil-β-CD complex; (d) minoxidil-β-CD physical mixture

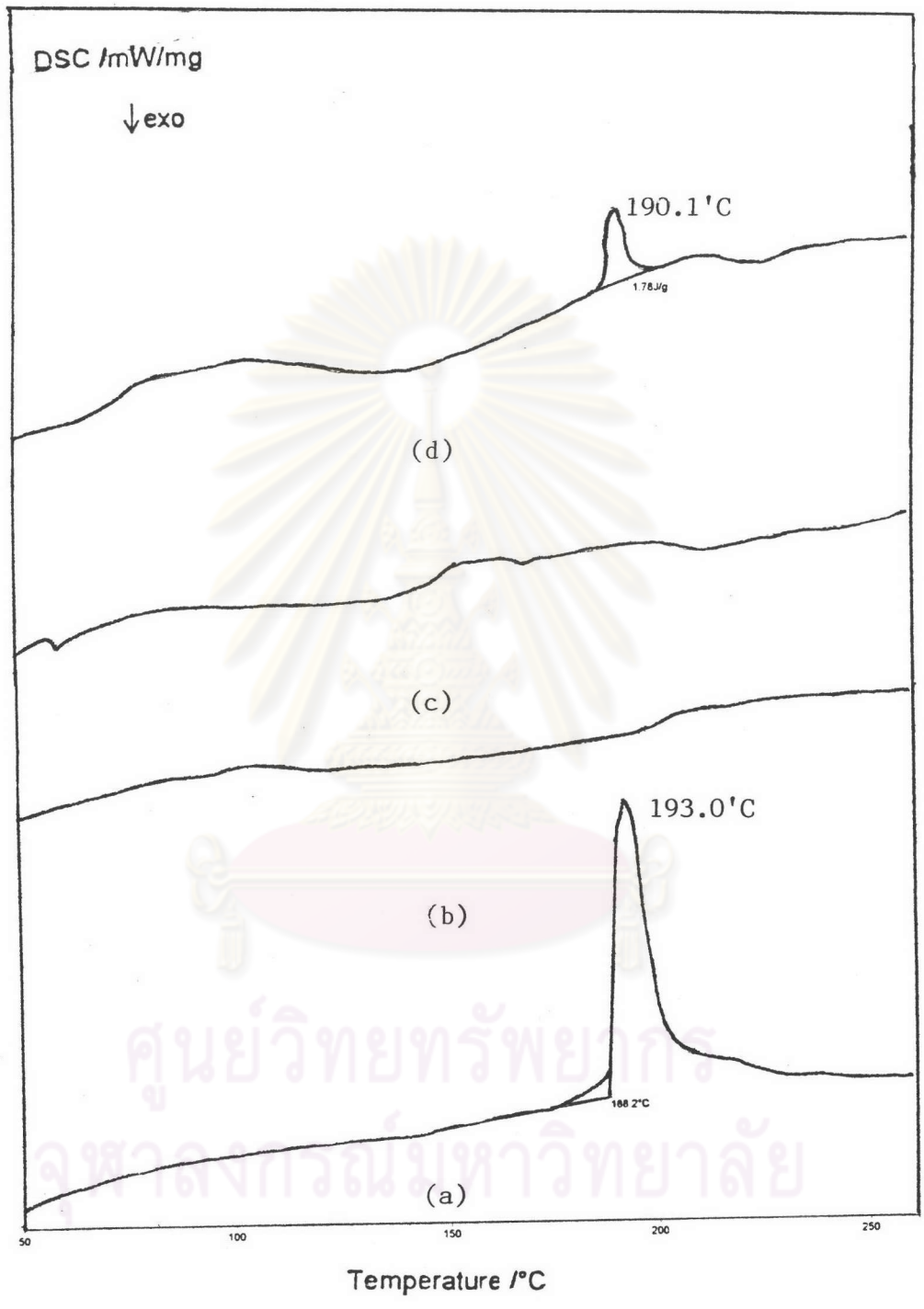


Figure 9. The DSC thermograms of (a) minoxidil; (b) HP-β-CD; (c) minoxidil-HP-β-CD complex; (d) minoxidil-HP-β-CD physical mixture

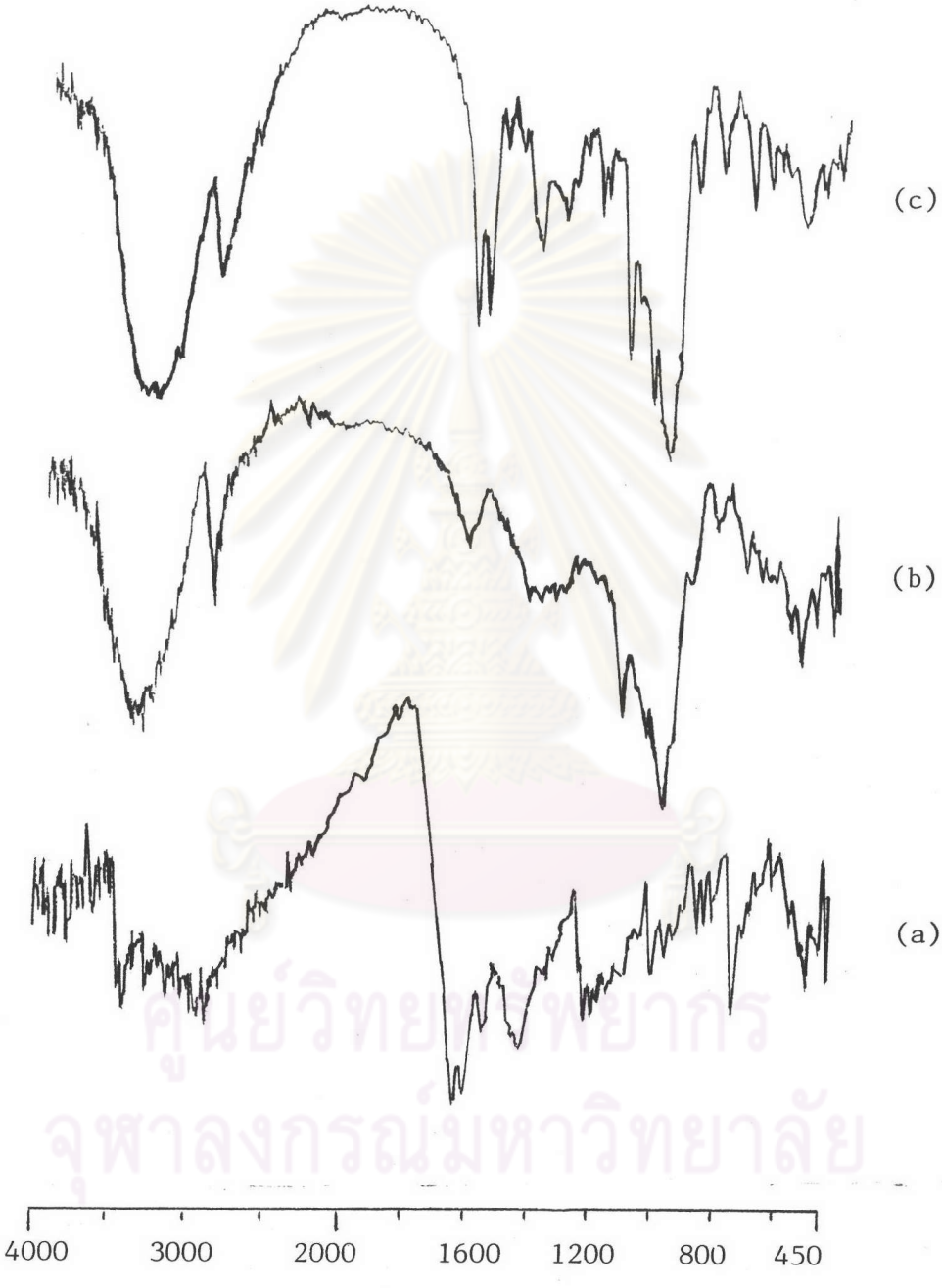


Figure 10. The IR spectra of (a) minoxidil; (b) β-CD; (c) minoxidil-β-CD complex

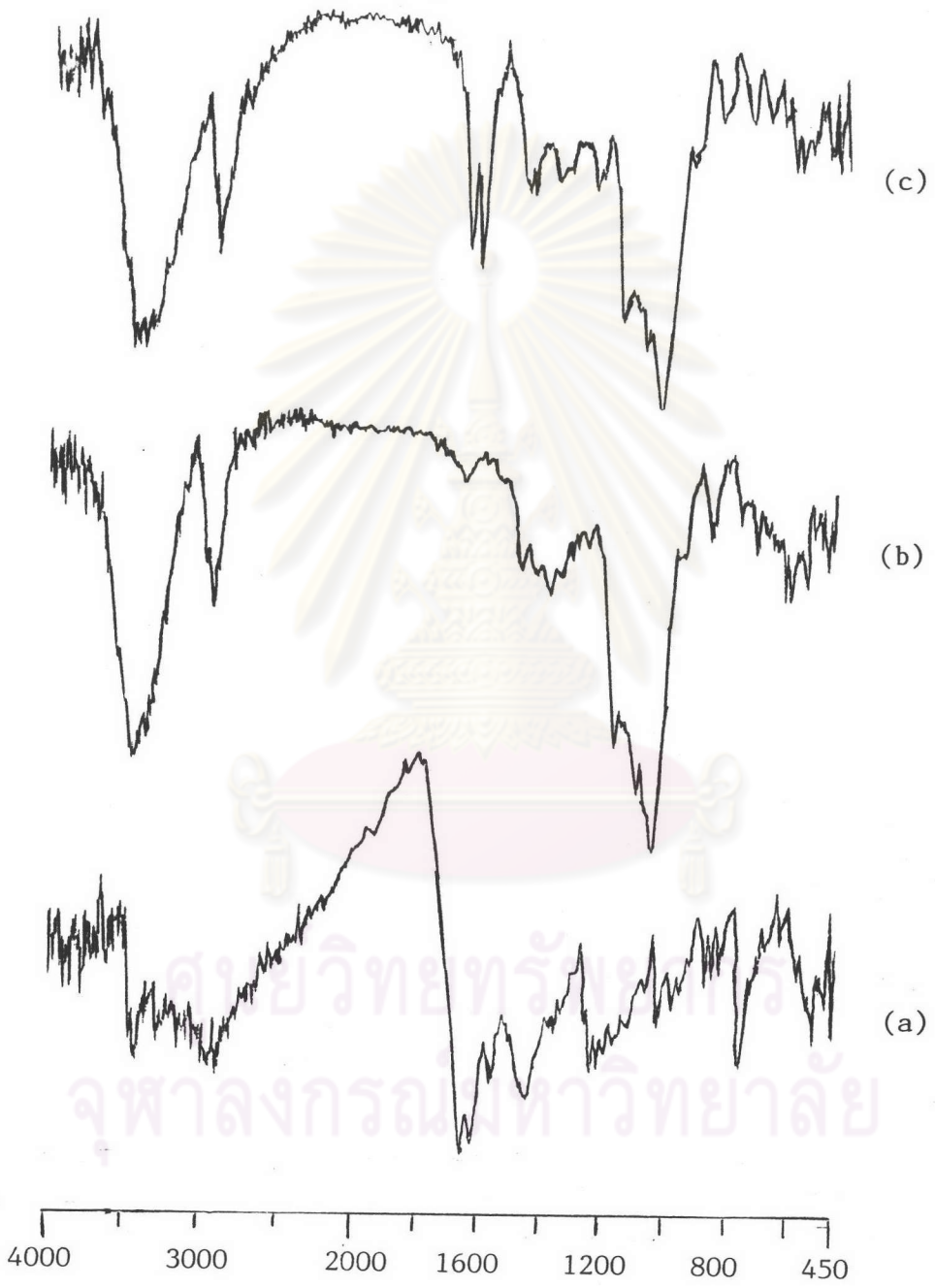


Figure 11. The IR spectra of (a) minoxidil; (b) HP-β-CD; (c) minoxidil-HP-β-CD complex

The IR spectra of both β-CD and HP-β-CD showed O-H stretching at 3600-3200 cm^{-1} (variable intensity), intermolecular hydrogen bonded OH at 3400-3200 cm^{-1} and C-O stretching at 1230-1000 cm^{-1}.

The IR spectra of both minoxidil-CDs complexes showed that O-H stretching (3600-3200 cm^{-1}) and C-O stretching (1230-1000 cm^{-1}) peaks were broader and more intense. This indicated that minoxidil and CD were held together in the complex by hydrogen bonding which supported the concept of the inclusion complex that formed by hydrogen bonding (Szeijtli, 1982). In addition, the IR spectra of both complexes still showed N-H in plane bending (1650-1590 cm^{-1}) and N-H out-of-plane bending (900-650 cm^{-1}) of the pyrimidine ring. This indicated that the piperidine ring of minoxidil might be included in CD's cavity.

From the above data, it can be concluded that minoxidil formed complexes with β-CD and HP-β-CD with some parts of its structure were included in CD's cavity.

4. Formulation of minoxidil solutions

The proportions of solvent composition used in the formulation of 2% minoxidil solution were shown in Table 2. In case of minoxidil solutions containing β-CD 0.1 %w/v, the suitable ratio of propylene glycol : ethanol : purified water was 20 : 40 : 40, and in minoxidil solutions containing HP-β-CD 5%w/v, the selected ratio was 20 : 30 : 50. Consequently, minoxidil solutions in the further study were formulated using these cosolvent systems as described.

Since β-CD could not dissolve all minoxidil in the solutions, propylene glycol and ethanol were used as cosolvents in the formulation. In addition, propylene glycol also held minoxidil in solution after evaporation of ethanol and water (Chiang et al., 1989b). Ethanol role has been primarily to solubilize minoxidil, other are used

Table 2. Solvent compositions of minoxidil solutions containing propylene glycol, ethanol USP and purified water* and solubility characteristics after storage under various conditions.

a. Minoxidil solutions containing β-CD 0.1% w/v

Propylene glycol (%v/v)	Ethanol USP (%v/v)	Result	
		Room temperature (48 hours)	Refrigerator (48 hours)
10	20	ppt	ppt
	30	ppt	ppt
	40	cs	cs
	50	cs	cs
20	20	ppt	ppt
	30	cs	ppt
	40	cs	cs
	50	cs	cs
30	20	cs	cs
	30	cs	cs
	40	cs	cs
	50	cs	cs

b. Minoxidil solutions containing HP-β-CD 5% w/v

Propylene glycol (%v/v)	Ethanol USP (%v/v)	Result	
		Room temperature (48 hours)	Refrigerator (48 hours)
10	20	ppt	ppt
	30	cs	ppt
	40	cs	cs
	50	cs	cs
20	20	ppt	ppt
	30	cs	cs
	40	cs	cs
	50	cs	cs
30	10	ppt	ppt
	20	cs	cs
	30	cs	cs
	40	cs	cs

* = purified water was added to volume
ppt = precipitation
cs = clear solution

et al., 1989b). Ethanol role has been primarily to solubilize minoxidil, other are used to enhance the permeation of minoxidil from formulation solutions (Tata, Weiner, and Flynn, 1994).

From the preliminary study, there was no statistical difference in stability of minoxidil in solutions containing EDTA sodium in concentrations varied at 0, 0.01, 0.05, 0.1 %w/v when the solutions were filled and sealed in 2-ml amber glass ampoules and stored in a hot air oven at 70°C for 2 weeks (Appendix II and III). The color of the solutions containing no EDTA sodium (0 %w/v) was more yellowlish than others, while the color of other solutions containing various amounts of EDTA sodium were not different. Therefore, EDTA sodium 0.01 %w/v was used as a chelating agent in the solutions.

5. Effects of CDs on minoxidil solutions

Four minoxidil solutions formulated with 0, 0.1, 0.4 and 0.7 %w/v of β-CD (Rx 2-5) and five formulations with 0, 5.0, 10.0, 15.0 and 20.0 %w/v of HP-β-CD (Rx 6-10) were prepared. The well known commercial product, Regaine® (Rx 1) was also used as the comparing formulation in the in vitro permeation studies. All formulations in the study were shown in Table 3.

The HPLC system used in this present study was accommodated from the method of Asmus et al. (1984) and of the USP XXII / NF XVII that used sodium dioctylsulfosuccinate as an ion paring reagent in the mobile phase. The HPLC chromatograms of the standard solutions and their calibration curve were shown in Figure 12 and 13, respectively.

Table 3. The formulae of minoxidil solutions containing various concentrations of CDs.

a. Minoxidil solution formulae containing β-CD

Rx	Minoxidil % w/v	β-CD % w/v	EDTA sodium % w/v	Propylene glycol % v/v	Ethanol USP % v/v	Purified water % v/v
1**	2	*	*	20	60	20
2	2	0	0.01	20	40	40
3	2	0.1	0.01	20	40	40
4	2	0.4	0.01	20	40	40
5	2	0.7	0.01	20	40	40

* no information.

** commercial solution, Regaine ®

b. Minoxidil solution formulae containing HP-β-CD

Rx	Minoxidil % w/v	HP-β-CD % w/v	EDTA sodium % w/v	Propylene glycol % v/v	Ethanol USP % v/v	Purified water % v/v
6	2	0	0.01	20	30	50
7	2	5	0.01	20	30	50
8	2	10	0.01	20	30	50
9	2	15	0.01	20	30	50
10	2	20	0.01	20	30	50

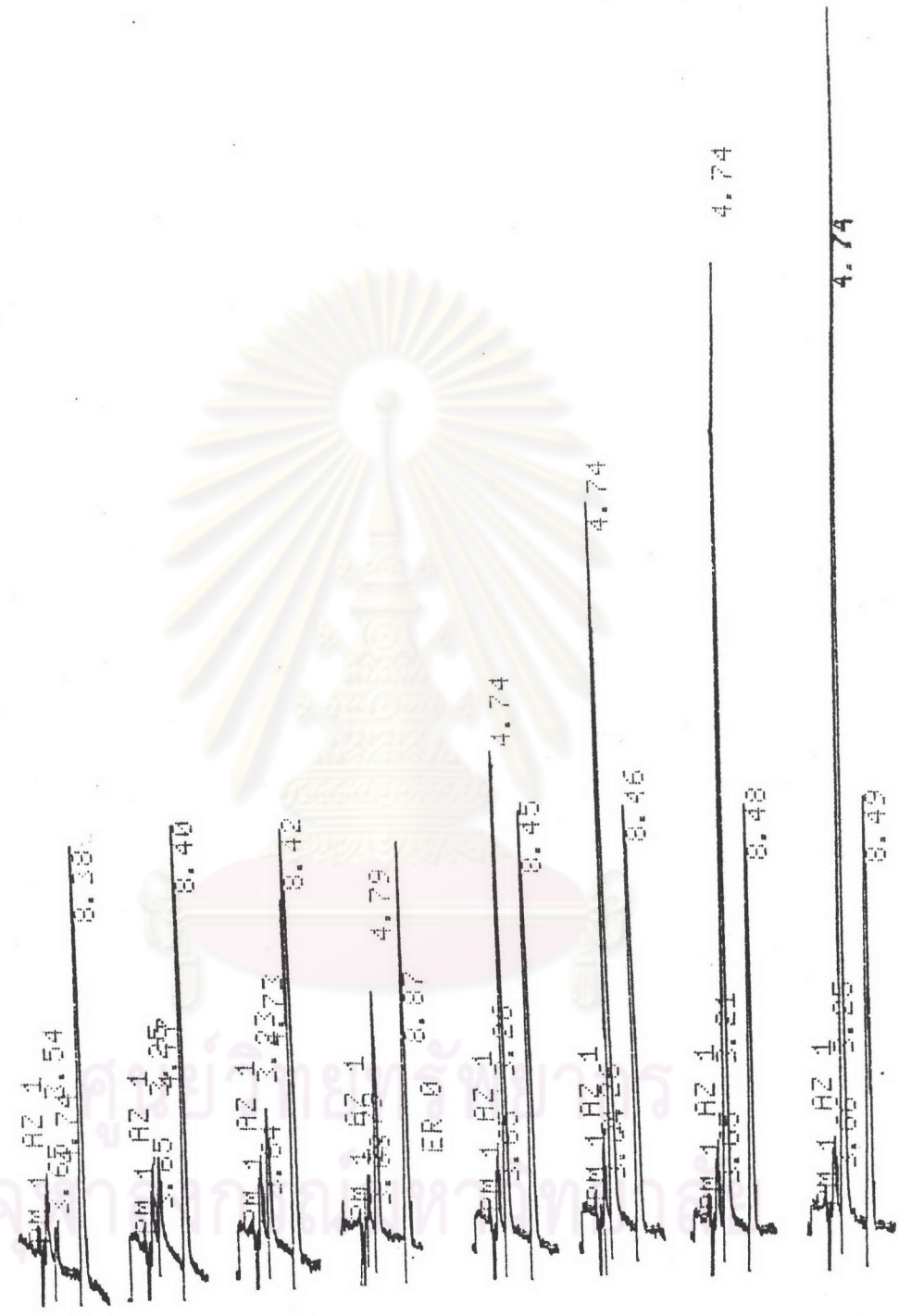


Figure 12. The HPLC chromatograms of the standard solutions of minoxidil (4.73-4.79 min); internal standard (8.38-9.13 min)

Data for calibration curve of standard solutions of minoxidil

concentration (mcg/ml)	Peak area ratio	inverse concentration	% recovery
0.0201	0.0554	0.0211	104.97
0.0603	0.1535	0.0599	99.34
0.1005	0.2444	0.0958	95.32
0.2010	0.5218	0.2053	102.14
0.4020	1.0265	0.4046	100.65
0.6030	1.5097	0.5954	98.74
0.8040	2.0583	0.8120	100.99
1.0050	2.5386	1.0017	99.67

r^2 = 0.9998

Mean = 100.23

SD = 2.78

%CV = 2.77

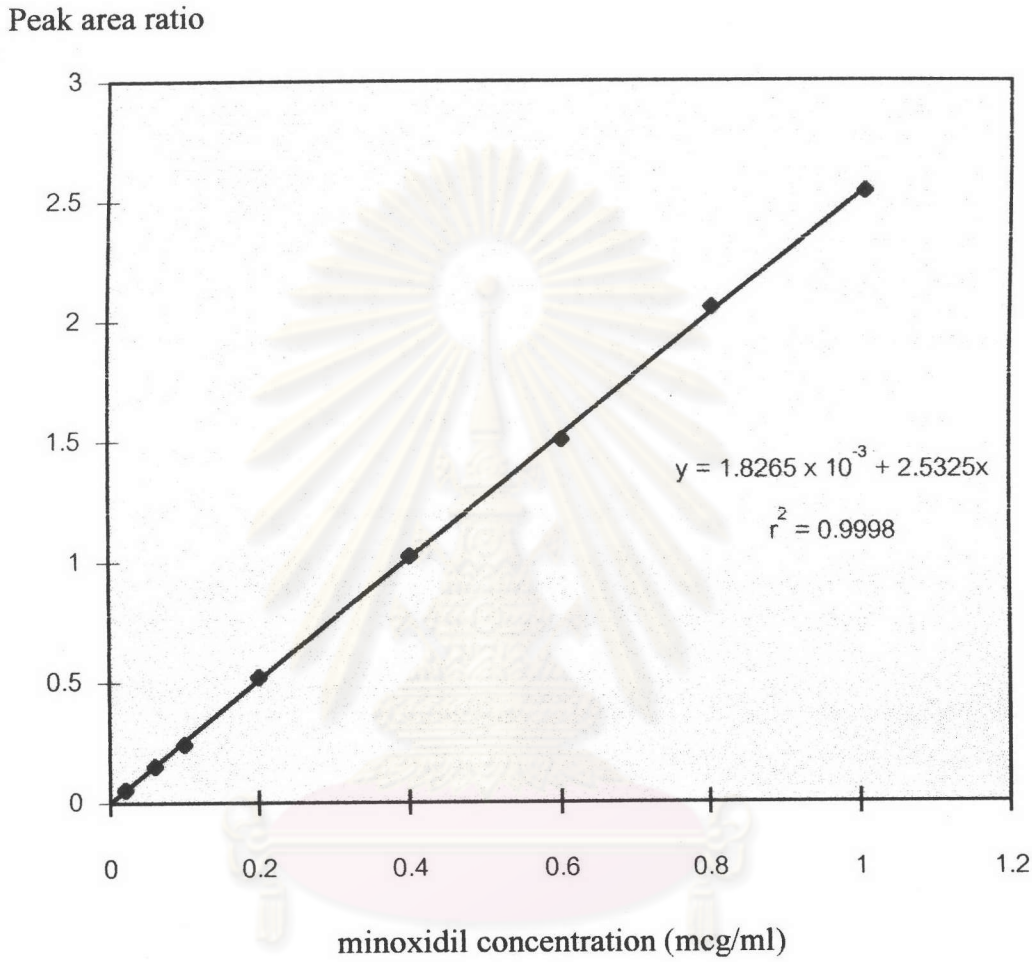


Figure 13. The calibration curve of standard solutions of minoxidil

5.1 Stability studies

The stability data of minoxidil solutions containing various concentrations of CDs (Table 3) were shown in Appendix II. From the stability data of each formula, least square method was used to obtain the straight line of the relationship between time and concentration of minoxidil remaining (ln $conc^n$) at room temperature and at 70°C.

Degradation rate constant (k) which was the slope (b) of each line was shown in Table 4. Since the slope are close to zero, their significance are tested using the t test with the null hypothesis $H_o : \beta = 0$ versus the alternative hypothesis $Ha : \beta \neq 0$ as follows

$$t_{0.001} = \frac{b}{S.E.(b)}, df = n-2 \qquad (11)$$

where β = population slope

b = a sample estimate of true slope

S.E. = standard error of slope

The t statistics of each formula (data not shown) was less than the confidential limit ($t_{0.001}$, df = 5 was 6.859). That is the null hypothesis $H_o : \beta = 0$ cannot be rejected; the slope was statistically not different from zero. Consequently, from the statistical point of view there was no significant degradation during the period of study.

Also, one way ANOVA of stability data of each formula (Appendix III) shows that there was no statistical difference in concentrations of minoxidil remaining at different times of storage. Thus, it can be concluded that concentration

Table 4. Degradation rate constants (k) at room temperature and at 70°C of minoxidil solutions formulae Rx 2-10.

Rx	Degradation rate constant (k) (mg/ml/day x 10^4)	
	At room temperature (30°C)	At 70°C
2	-3.5714	-5.6122
3	-5.6122	-5.3571
4	1.2755	-6.3775
5	-2.5510	-2.5510
6	-12.5000	-6.3775
7	-7.3980	-4.3367
8	-3.3136	1.5306
9	-4.8469	2.5510
10	-4.3367	1.5306

of minoxidil remaining in each formula did not change throughout the study (84 days).

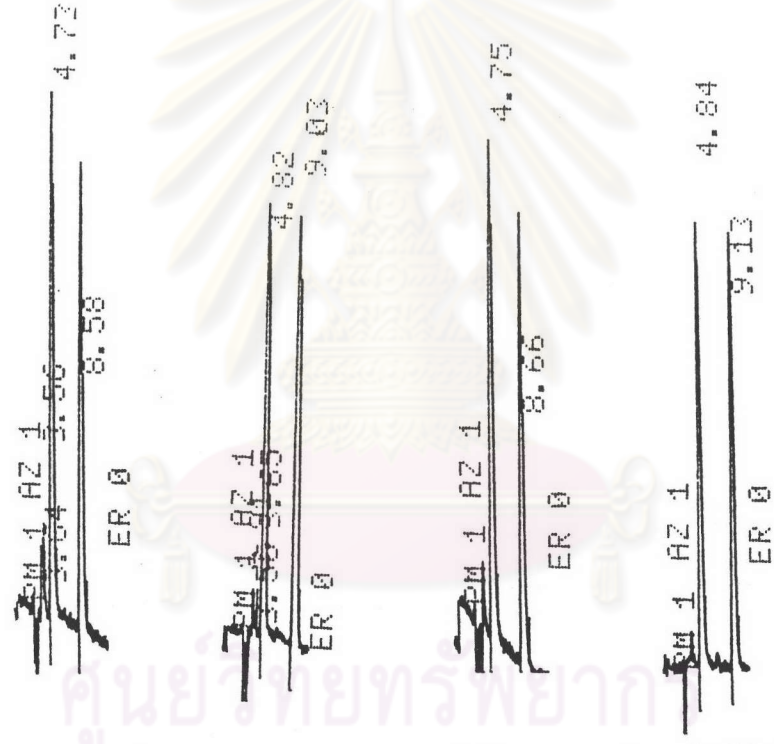
The color of minoxidil solutions in all formulations kept at room temperature did not change throughout the study. This was consistent with a previous study by Pimolpan Pithayanukul (1988) that EDTA sodium 0.01% could stop the color change of the minoxidil solutions when kept at room temperature for 40 days.

However, the color of the solutions kept at 70°C turned into 'yellow' after about 2 weeks regardless of whether solutions containing any CDs or not. Hence, a chelating agent, EDTA sodium, and also CDs could not stop the discoloration reaction at high temperature.

Two studies of Cramer, Saenger, and Spatz (1967) and Ukema et al. (1985) reported that the complex stability constant or complex formation constant (Kc) decreased rapidly with increasing temperature due to dissociation of the complex. Therefore, it may be said that at high temperature as in this study, there was dissociation of the minoxidil-CDs complexes so that free minoxidil was exposed to the degradation or discoloration reaction.

The results of this study comply with those of Haines-Nutt, Adams, and Bendell (1984) that minoxidil solutions developed a slight yellow coloration after 3 months at room temperature, but no chemical degradation was seen; the assay results using HPLC method remained constant. The HPLC chromatograms of sample solutions at 70°C (Figure 14) showed that the chromatograms of yellow solutions in this study did not different from of the standard solutions (Figure 12).

In addition, the results from thin layer chromatography (TLC) (Appendix IV) showed that Rf values of minoxidil in solutions at room temperature and at 70°C were not different, and any other spots were not seen in solutions at 70°C



(a) (b) (c) (d)

Figure 14. The HPLC chromatograms of the sample solutions of minoxidil (4.73-4.79 min); internal standard (8.38-9.13 min)

(a) with β-CD at room temperature (c) with β-CD at 70°C

(b) with HP-β-CD at room temperature (d) with HP-β-CD at 70°C

in spite of the discoloration of the solutions. It may be said that the colored product had so small extent that it could not be detected by TLC method. However, the UV spectrophotometric scanning from 200-700 nm. of minoxidil solutions kept at room temperature and at 70°C (Appendix V) showed that there was a slight difference that it might due to the yellow coloration of the solution at high temperature.

There were few studies on the stability of minoxidil in solutions and the exact mechanisms of the discoloration reaction between minoxidil and propylene glycol has not been proved. In order to investigate more about any effects of CDs on minoxidil stability, the further study in this discoloration reaction must be done.

5.2 In vitro permeation studies

In vitro permeation studies were done to investigate the effects of CDs on permeation of minoxidil through newborn pig skin throughout 24 hours. In this study, the permeation of minoxidil was evaluated in newborn pig skin since it is remarkably similar to human skin and can be used to model human skin properties (Bisset and Mc Bride, 1983). Hawkins and Reifenrath (1986) found good correlation between the diffusion through in vitro pig skin and in vivo human skin.

The receptor phase in this study was isotonic phosphate buffer pH 7.4 which has frequently been used since it mimics the biological fluid.

Data of individual permeation run were given in Appendix VI. An extrapolation of the pseudo-steady state portion of the plot of cumulative amount of minoxidil per unit area versus time to the intercept on the time axis provides the lag time (t_L). Steady state conditions prevail after approximately 2.7 times the lag time (Barry, 1983).

The steady state flux (Jss) was the slope of the plot of cumulative amount of minoxidil per unit area versus time. The normalized flux of each permeation run was then calculated by multiplying the flux with $h / \bar{h}$, where h was the membrane thickness of each permeation run, $\bar{h}$ was the average membrane thickness of all membrane used in the study. The average normalized flux of each solution formula was used to determine the permeability of minoxidil from each formula.

Percent cumulative amounts of minoxidil in receiver compartment at 12 and 24 hours were also used to compare the total amount of minoxidil that could permeate through membrane. The data were shown in Table 5 and Figure 15-16.

5.2.1 The average normalized flux

In β-CD group (Rx 2-5), there was no statistical difference in average normalized flux of formulae containing β-CD 0.1, 0.4 or 0.7 %w/v (Rx 3-5) or the commercial solution (Rx 1). The flux of the formula containing no β-CD and ethanol 40 %v/v (Rx 2) was significantly highest in the group (Table 6).

In HP-β-CD group (Rx 6-10), the fluxes of formulae containing HP-β-CD 0, 5, 10 and 15 %w/v (Rx 6-9, respectively) were significantly higher than the formula containing HP-β-CD 20 %w/v (Rx 10). The flux of the formula containing no HP-β-CD and ethanol 30 %v/v (Rx 6) was statistically higher than the commercial solution (Rx 1). However, there was no statistical difference between the latter solution and other formulae with HP-β-CD (Table 7).

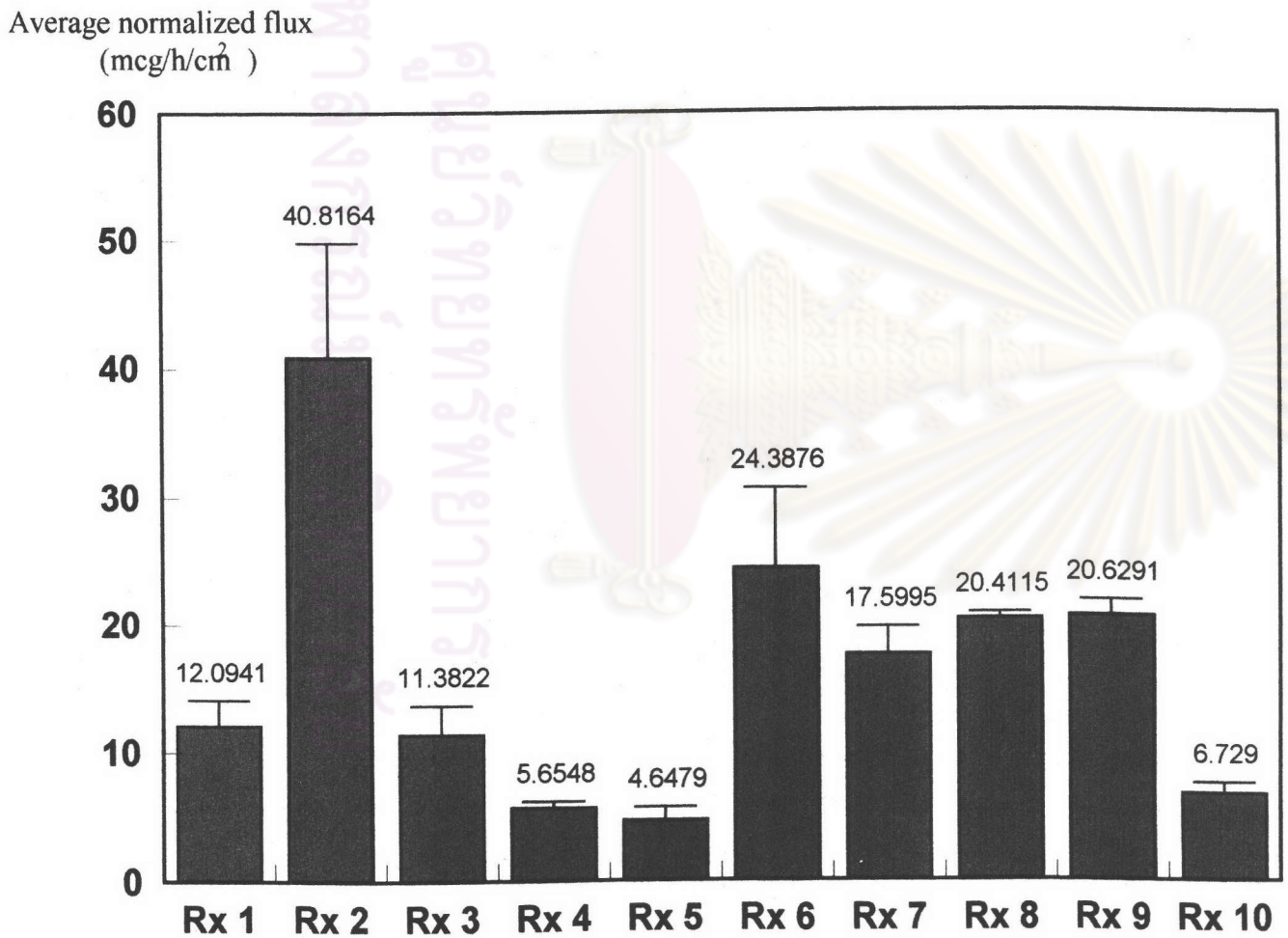
Duncan's new multiple range test of average normalized flux between ten formulations which were β-CD group (Rx 2-5), HP-β-CD group (Rx 6-10) and the commercial solution (Rx 1) was also used to verify the difference among all formulae and shown in Table 8.

Table 5. Average normalized fluxes of minoxidil through newborn pig skin and percent cumulative amounts of minoxidil in receiver compartment.

Rx	Average normalized flux* ($mcg/h/cm^2$)	Percent cumulative amounts of minoxildil in receiver compartment*	
		12 hours	24 hours
1	12.0941 ± 3.4633	3.66 ± 1.36	4.77 ± 1.90
2	40.8164 ± 15.4412	11.11 ± 4.83	13.35 ± 6.40
3	11.3822 ± 3.9166	3.80 ± 0.75	5.83 ± 0.78
4	5.6548 ± 0.8183	1.63 ± 0.64	2.69 ± 0.86
5	4.6479 ± 1.7815	1.31 ± 0.68	1.98 ± 1.00
6	24.3876 ± 10.8341	10.49 ± 5.69	16.82 ± 10.28
7	17.5995 ± 3.6931	10.15 ± 2.79	14.75 ± 5.37
8	20.4115 ± 0.7393	10.75 ± 1.07	17.89 ± 1.88
9	20.6291 ± 2.0792	8.11 ± 0.69	10.77 ± 2.52
10	6.7290 ± 1.4324	2.80 ± 1.50	4.68 ± 3.25

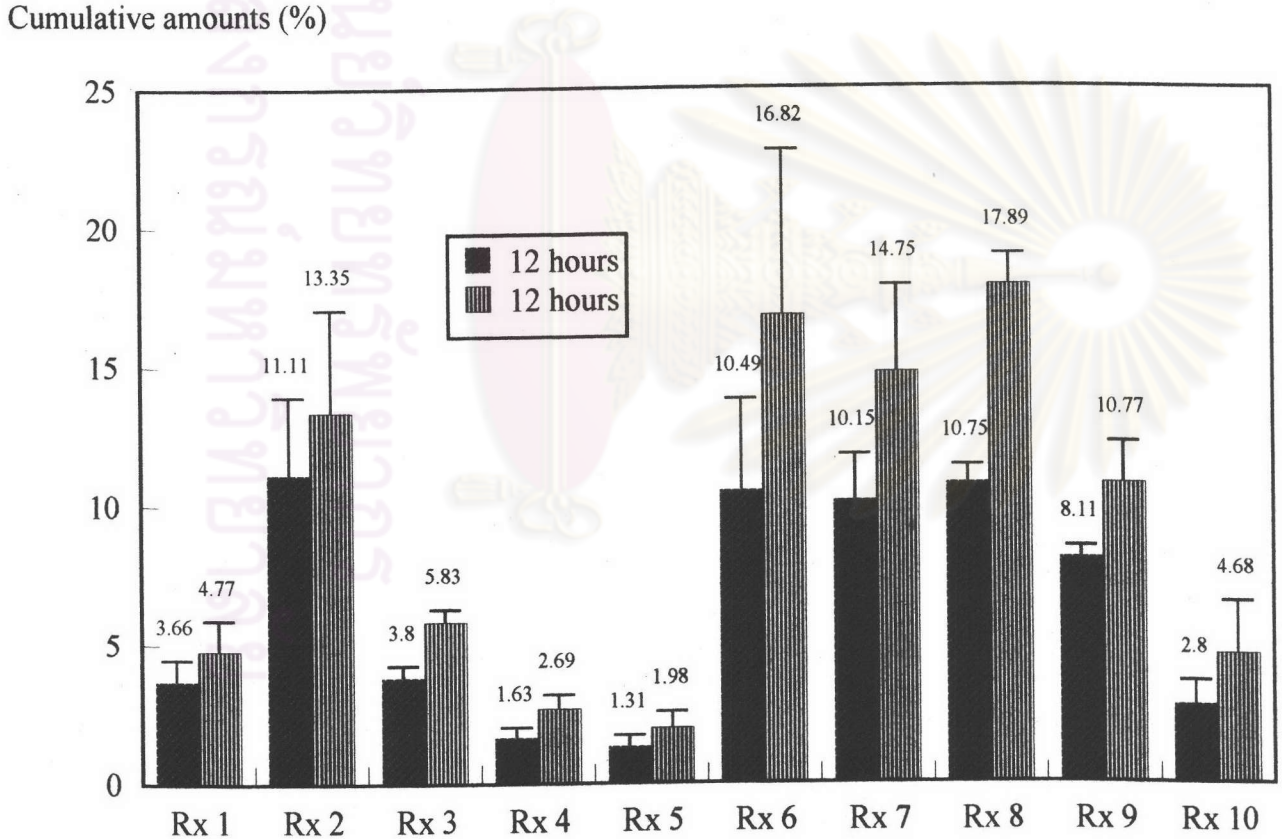
* = mean ± SD , n = 3

Figure 15. Average normalized fluxes* of minoxidil through newborn pig skin



* = Mean ± SE

Figure 16. Percent cumulative amounts* of minoxidil in receiver compartment at 12 hours and 24 hours



* = Mean ± SE

Table 6. Duncan's new multiple range test of average normalized fluxes of minoxidil from the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of β-CD (Rx 2-5) through newborn pig skin.

Rx	5	4	3	1	2
Average normalized flux ($mcg/h/cm^2$)	4.6749	5.6548	11.3822	12.0941	40.8164

Rx	1	2	3	4	5
1		×	-	-	-
2	×		×	×	×
3	-	×		-	-
4	-	×	-		-
5	-	×	-	-	

× = Statistical difference ($p<0.05$)

\- = Not statistical difference ($p>0.05$)

Table 7. Duncan's new multiple range test of average normalized fluxes of minoxidil from the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of HP-β-CD (Rx 6-10) through newborn pig skin.

Rx	10	1	7	8	9	6
Average normalized flux ($mcg/h/cm^2$)	6.7290	12.0941	17.5995	20.4115	20.6291	24.3876

Rx	1	6	7	8	9	10
1		×	-	-	-	-
6	×		-	-	-	×
7	-	-		-	-	×
8	-	-	-		-	×
9	-	-	-	-		×
10	-	×	×	×	×	

× = Statistical difference ($p<0.05$)

\- = Not statistical differece ($p>0.05$)

Table 8. Duncan's new multiple range test of average normalized fluxes of minoxidil from minoxidil solutions formulae Rx 1-10 through newborn pig skin.

Rx	5	4	10	3	1	7	8	9	6	2
Average normalized flux ($mcg/h/cm^2$)	4.6479	5.6548	6.7290	11.3822	12.0941	17.5995	20.4115	20.6291	24.3876	40.8164

Rx	1	2	3	4	5	6	7	8	9	10
1		×	-	-	-	×	-	-	-	-
2	×		×	×	×	×	×	×	×	×
3	-	×		-	-	×	-	-	-	-
4	-	×	-		-	×	-	×	×	-
5	-	×	-	-		×	×	×	×	-
6	×	×	×	×	×		-	-	-	×
7	-	×	-	-	×	-		-	-	-
8	-	×	-	×	×	-	-		-	×
9	-	×	-	×	×	-	-	-		×
10	-	×	-	-	-	×	-	×	×	

× = Statistical difference ($p<0.05$)

\- = Not statistical difference ($p>0.05$)

For the formulae containing no CD (Rx 2,6), their fluxes were more than other formulae containing different concentrations of CD (Rx 3-5, 7-10). The flux of formula Rx 2 was significantly highest, while the flux of formula Rx 6 was significantly higher than the fluxes of formulae containing various concentrations of β-CD (Rx 3-5).

Effect of alcohol on permeation of minoxidil was seen in formulation Rx 2 (ethanol 40 %v/v) and Rx 6 (ethanol 30 %v/v) that the flux of the former formula was significantly higher than the latter one. This result is consistent with the study of Tata, Weiner, and Flynn (1994) that more minoxidil penetrated the skin as the proportion of ethanol in the solutions was increased. But the same effect was not seen in a commercial solution (Rx 1) containing ethanol 60 %v/v.

Effects of different types of CD on permeation of minoxidil were seen in the formulae containing β-CD (Rx 3-5) and the formulae containing HP-β-CD (Rx 7-10). The fluxes of the formulae Rx 7-9 were significantly higher than those of formulae containing β-CD 0.7 %w/v (Rx 5), but were not statistical different from the formula containing β-CD 0.1 %w/v (Rx 3). Only the fluxes of formulae Rx 8 and 9 were significantly higher than that of formula Rx 4. The more HP-β-CD in the formula (Rx 10) was the more its flux decreased, similar to the formulae containing β-CD (Rx 3-5).

These results indicated that β-CD and HP-β-CD decreased the flux of minoxidil through newborn pig skin. This may due to an increment in hydrophilicity of minoxidil in the solution by CD complexation.

The similarity was mentioned in the following studies. Loftsson, Frioriksdottir, Thorisdottir et al. (1994) revealed that maximum penetration of acetazolamide through a semi-permeable membrane was obtained when just enough HP-β-CD was used to keep all the drug in solution. An addition of too much CD or

addition of CD to aqueous eye-drop preparations containing water soluble drugs will reduce the drug availability due to their very hydrophilic character and large effective radii (Loftsson, Frioriksdottir, Thorisdottir et al., 1994).

Another study by Loftsson, Frioriksdottir, Ingvarsdottir et al. (1994) also reported that HP-β-CD increased the flux of hydrocortisone through a semi-permeable membrane until all hydrocortisone had dissolved, after that the flux decreased with increasing HP-β-CD concentrations. A maximum flux was obtained when just enough HP-β-CD was used to dissolve all the drug in the aqueous vehicle.

Since the donor phase in this study was clear solution, therefore the effects of CDs in increasing minoxidil flux were not seen. However, precipitation of minoxidil on permeating membrane was not seen in formulae containing CD (Rx 3-5, 7-10). Conversely, precipitation of minoxidil was seen after 12 hours in formulae not containing any CD (Rx 1, 2 and 6). Thus, both CDs in this study increased the solubility of minoxidil in aqueous solution by complex formation. More soluble of minoxidil-CD complexes resulted in more hydrophilicity. Consequently, the decrement in minoxidil flux was shown in formulae containing CD.

5.2.2 Percent cumulative amount of minoxidil at 12 hours

Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 12 hours were shown in Table 9-11. The comparison of β-CD group (Rx 2-5) to the commercial solution (Rx 1) showed that percent cumulative amounts of minoxidil at 12 hours of formulae containing β-CD 0.1, 0.4 or 0.7 %w/v (Rx 3-5, respectively) and the commercial solution (Rx 1) was statistically lower than the formula containing no β-CD and ethanol 40 %v/v (Rx 2) (Table 9). This result complied with those found in the flux.

Table 9. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 12 hours of the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of β-CD (Rx 2-5).

Rx	5	4	1	3	2
Cumulative amount (%)	1.31	1.63	3.66	3.80	11.11

Rx	1	2	3	4	5
1		×	-	-	-
2	×		×	×	×
3	-	×		-	-
4	-	×	-		-
5	-	×	-	-	

× = Statistical difference ($p<0.05$)

\- = Not statistical difference ($p>0.05$)

Table 10. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 12 hours of the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of HP-β-CD (Rx 6-10).

Rx	10	1	9	7	6	8
Cumulative amount (%)	2.80	3.66	8.11	10.15	10.49	10.75

Rx	1	6	7	8	9	10
1		×	×	×	-	-
6	×		-	-	-	×
7	×	-		-	-	×
8	×	-	-		-	×
9	-	-	-	-		×
10	-	×	×	×	×	

× = Statistical difference ($p<0.05$)

- = Not statistical difference ($p>0.05$)

In HP-β-CD group (Rx 6-10) and the commercial solution (Rx 1), percent cumulative amounts at 12 hours of formulae containing HP-β-CD 0, 5 and 10 %w/v (Rx 6-8) were significantly higher than that of formula Rx 1. Adding too much of HP-β-CD (Rx 10) resulted in decreasing of percent cumulative amounts to the same amount as formula Rx 1. While the percent cumulative amounts of formulae containing HP-β-CD 5 and 10 %w/v (Rx 7,8) were significantly higher than that of the formula Rx 1, but their fluxes were not statistically different (Table 7).

Duncan's new multiple range test of all formulations were displayed in Table 11. The percent cumulative amounts at 12 hours of formulae containing no β-CD and ethanol 40 %v/v (Rx 2) was not statistically different from the formula containing no HP-β-CD and ethanol 30 %v/v (Rx 6) or formulae containing HP-β-CD 5, 10 or 15 %v/v (Rx 7-9). This result which was different from their fluxes (Table 8) was due to precipitation of minoxidil on permeating membrane causing a decrement in the amount of minoxidil that could be absorbed (Tsai, Cappel et al., 1991; Tsai, Flynn et al., 1993).

Tsai, Cappel et al. (1991) and Tsai, Flynn et al. (1993) explained that the precipitation limited the amount of minoxidil that could be absorbed and led to poor percutaneous absorption of drug from the formulation.

Table 11 also showed that percent cumulative amounts of minoxidil in formulae of HP-β-CD group (Rx 7-9) were more than the formulae of β-CD group (Rx 3-5). This could be explained that HP-β-CD was able to increase the solubility of minoxidil more than β-CD with respect to the results from solubility studies. Consequently, the amounts of minoxidil that could permeate from formulae containing HP-β-CD were more than those from formulae containing β-CD. In addition, the decrement in percent cumulative amounts of minoxidil in the formula containing HP-β-CD 20 %w/v (Rx 10) suggested that it was owing to its very hydrophilicity.

Table 11. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 12 hours of minoxidil solutions formulae Rx 1-10.

Rx	5	4	10	1	3	9	7	6	8	2
Cumulative amount (%)	1.31	1.63	2.80	3.66	3.80	8.11	10.15	10.49	10.75	11.11

Rx	1	2	3	4	5	6	7	8	9	10
1		×	-	-	-	×	×	×	-	-
2	×		×	×	×	-	-	-	-	×
3	-	×		-	-	×	×	×	-	-
4	-	×	-		-	×	×	×	×	-
5	-	×	-	-		×	×	×	×	-
6	×	-	×	×	×		-	-	-	×
7	×	-	×	×	×	-		-	-	×
8	×	-	×	×	×	-	-		-	×
9	-	-	-	×	×	-	-	-		×
10	-	×	-	-	-	×	×	×	×	

× = Statistical difference ($p<0.05$)

- = Not statistical difference ($p>0.05$)

5.2.3 Percent cumulative amount of minoxidil at 24 hours

Duncan's new multiple range test of percent cumulative amounts of minoxidil at 24 hours in β-CD group (Table 12) or in HP-β-CD group (Table 13) were not different from their Duncan's new multiple range test of percent cumulative amounts of minoxidil at 12 hours (Table 9, 10). Except for the formulae Rx 9 and Rx 10, their percent cumulative amounts were not statistically different at 24 hours.

Duncan's new multiple range test of percent cumulative amounts at 24 hours of all formulae were compared and shown in Table 14. It showed that percent cumulative amounts of the formula containing HP-β-CD 10 % w/v (Rx 8) was the highest (though not statistically significant), not the formula Rx 2 which had the highest flux.

From all data presented in the permeation studies, it revealed that CD did not increase the flux of minoxidil from solutions through newborn pig skin. As a result of an increment of minoxidil solubility in the solution by a complex formation, minoxidil did not precipitate out of the solution. Therefore, the amounts of minoxidil that could be absorbed through membrane were increased. On the other hand, adding too much of CD in the solution made more hydrophilic so that the permeation of minoxidil was decreased. The decrement of minoxidil permeation from the formulae containing β-CD was more evident than from the formulae containing HP-β-CD. The reason was that dissociation of minoxidil-β-CD complex was more difficult than that of minoxidil-HP-β-CD complex owing to the higher formation constant (Kc) shown in solubility studies.

Table 12. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 24 hours of the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of β-CD (Rx 2-5).

Rx	5	4	1	3	2
Cumulative amount (%)	1.98	2.69	4.77	5.83	13.35

Rx	1	2	3	4	5
1		×	-	-	-
2	×		×	×	×
3	-	×		-	-
4	-	×	-		-
5	-	×	-	-	

× = Statistical difference ($p<0.05$)

\- = Not statistical difference ($p>0.05$)

Table 13. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 24 hours of the commercial minoxidil solution, Regaine® (Rx 1) and minoxidil solutions containing various concentrations of HP-β-CD (Rx 6-10).

Rx	10	1	9	7	6	8
Cumulative amount (%)	4.68	4.77	10.77	14.75	16.82	17.89

Rx	1	6	7	8	9	10
1		×	×	×	-	-
6	×		-	-	-	×
7	×	-		-	-	×
8	×	-	-		-	×
9	-	-	-	-		-
10	-	×	×	×	-	

× = Statistical difference ($p<0.05$)

- = Not statistical difference ($p>0.05$)

Table 14. Duncan's new multiple range test of percent cumulative amounts of minoxidil in receiver compartment at 24 hours of minoxidil solutions formulae Rx 1-10.

Rx	5	4	10	1	3	9	2	7	6	8
Cumulative amount (%)	1.98	2.69	4.68	4.77	5.83	10.77	13.35	14.75	16.82	17.89

Rx	1	2	3	4	5	6	7	8	9	10
1		×	-	-	-	×	×	×	-	-
2	×		-	×	×	-	-	-	-	×
3	-	-		-	-	×	×	×	-	-
4	-	×	-		-	×	×	×	-	-
5	-	×	-	-		×	×	×	×	-
6	×	-	×	×	×		-	-	-	×
7	×	-	×	×	×	-		-	-	×
8	×	-	×	×	×	-	-		-	×
9	-	-	-	-	×	-	-	-		-
10	-	×	-	-	-	×	×	×	-	

× = Statistical difference ($p<0.05$)

- = Not statistical difference ($p>0.05$)