CHAPTER IV

RESULTS AND DISCUSSION

Formulation of 1% w/w Clindamycin Hydrochloride Gel.

Generally, the formula of a medicated gel preparation is composed of a drug or drugs, gelling agents, humectants, preservatives and/or other stabilizers and vehicles. From a preliminary study, carbopols 940 and 941 gave an opaque gel due to the incompatibility of drug and gelling agent whereas poloxamer 188 expressed a very sticky gel. Therefore, the carbopols and poloxamer 188 were excluded for further study. The appropriate gelling agents for this formulation were poloxamer 407, hydroxyethyl cellulose and hydroxypropyl methylcellulose.

Physical appearances were studied to select a proper concentration of each gelling agent. The preparation containing 15, 18, 20 % w/w of poloxamer 407 resulted a clear, transparent and air bubble free gel. With increasing the concentration of poloxamer 407 to 20 % w/w, the increasingly sticky gel was observed. From the result in Table 1, the 18 % w/w of poloxamer 407 was selected to further study.

The formulation consisted of 1 % w/w hydroxyethyl cellulose was a liquid gel, whereas 3 % w/w concentration expressed a jelly-liked gel which was difficult to apply. Physical appearances of hydroxyethyl cellulose gel was shown in Table 2. The concentration of 2 % w/w was selected.

From the result in Table 3, the formulation consisted of 2 % w/w of hydroxypropyl methylcellulose was not rigid. Increasing the concentration resulted in a slightly opaque gel. The concentration of 3 % w/w of hydroxypropyl methylcellulose gave a proper gel.

Table 1. Physical Appearances of Clindamycin Hydrochloride Gel Prepared from Poloxamer 407 in Various Concentrations.

Concentration (% w/w)	Clarity [a]	Air Bubble	Sticky	Rigidity
15	-	-	++	-
18	-	-	+++	++
20	-	-	++++	+++

a : (+) = translucent, (-) = transparent
the number of the symbols of (+) and (-) showed a degree of the appearance and no appearance, respectively.

Table 2. Physical Appearances of Clindamycin Hydrochloride Gel Prepared from Hydroxyethyl Cellulose in Various Concentrations.

Concentration (% w/w)	Clarity[a]	Air Bubble	Sticky	Rigidty
1	-	-	+	-
2	-	-	++	++
3	-	+	++	+++

a : (+) = translucent, (-) = transparent
the number of the symbols of (+) and (-) showed a degree of the appearance and no appearance, respectively.

Table 3. Physical Appearances of Clindamycin Hydrochloride Gel Prepared from Hydroxypropyl Methylcellulose in Various Concentrations.

Concentration (% w/w)	Clarity[a]	Air Bubble	Sticky	Rigidity
2	-	-	-	-
3	+	+	+	+
4	++	++	++	++

a : (+) = translucent, (-) = transparent
the number of the symbols of (+) and (-) showed a degree of the appearance and no appearance, respectively.

Eventually, the formulations of 1 % w/w clindamycin hydrochloride gel are shown in Table 4. The formulations containing poloxamer 407, hydroxyethyl cellulose and hydroxypropyl methylcellulose as gelling agents were represented as Formulation I, II and III, respectively.

Analysis of Clindamycin Hydrochloride.

The chromatogram of clindamycin hydrochloride analysis using HPLC method is shown in Figure 4. The retention time of clindamycin hydrochloride and its internal standard, phenylethyl alcohol, were 7.5 and 4.5 minutes, respectively. The prepared gels and blank gels were run simultaneously to check for any interferance. Chromatograms, in Figure 4, 5, 6, 7, exhibited that there were no any other peaks interfered with both clindamycin hydrochloride and internal standard peak. Therefore, the calibration curve was used with all formulations.

The sample of clindamycin hydrochloride data and its corresponding calibration curve are shown in Table 5 and Figure 8, respectively. The coefficients of determination (r^2) of regression line is highly significant. (r^2 = 0.999)

Clindamycin hydrochloride concentrations estimated from the regression line are called "Inversely Estimated Concentrations", which are calculated to percentage by comparing them with their corresponding actual concentrations. These percentages are called "Percent Theory". The coefficient of variation is a parameter which indicates the variation of the variables from the fit line.

Table 4. The Formulations of 1 % w/w Clindamycin Hydrochloride Gels.

Formulation	I	II	III
		(%w/w)	
Clindamycin Hydrochloride	1	1	1
Glycerin	10	10	10
Poloxamer 407	18	-	-
Hydroxyethyl Cellulose	-	2	-
Hydroxypropyl Methylcellulose	-	-	3
Bronopol(R)	0.02	0.02	0.02
Acetate Buffer (pH 4.5) to	100	100	100

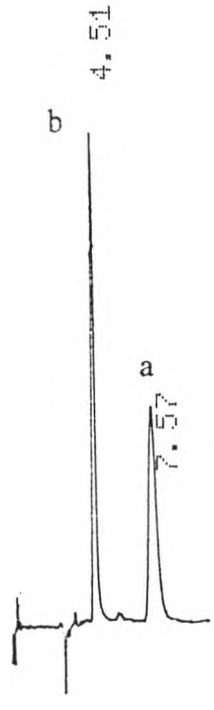

Figure 4 The HPLC Chromatogram of Clindamycin Hydrochloride (a) using Phenylethyl Alcohol (b) as Internal Standard.

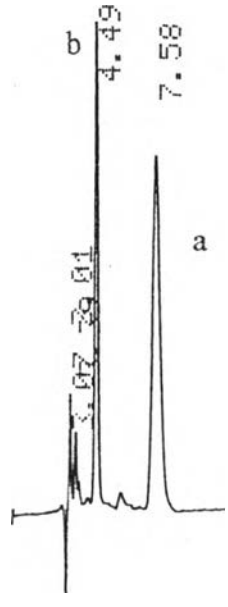

Figure 5 The HPLC Chromatogram of Clindamycin Hydrochloride in Poloxamer 407 Gel.

(a) Clindamycin Hydrochloride

(b) Phenylethyl Alcohol

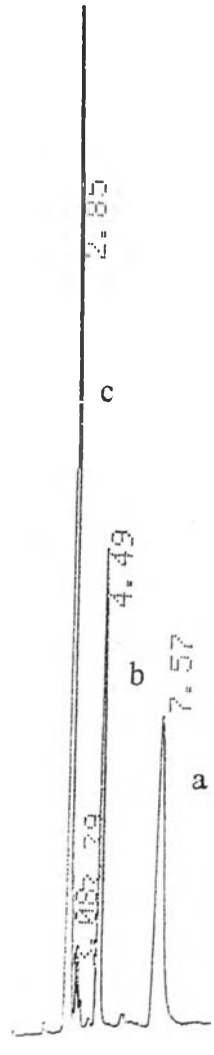

Figure 6 The HPLC Chromatogram of Clindamycin Hydrochloride in Hydroxyethyl Cellulose Gel.

(a) Clindamycin Hydrochloride

(b) Phenylethyl Alcohol

(c) Hydroxyethyl Cellulose

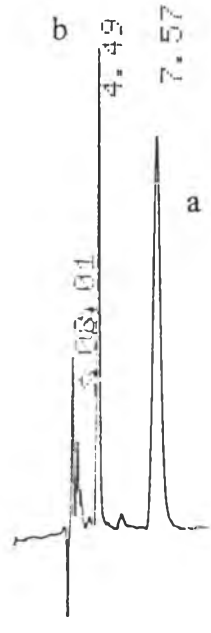

Figure 7. The HPLC Chromatogram of Clindamycin Hydrochloride in Hydroxypropyl Methylcellulose Gel.

(a) Clindamycin Hydrochloride

(b) Phenylethyl Alcohol

Table 5. Typical Standard Curve Data of Clindamycin Hydrochloride Concentrations Using Linear Regression.

Standard No.	Concentration (mcg/ml)	Peak Area Ratio	Inversely Estimated Concentration [a] (mcg/ml)	% Theory [b]
1	20	0.7920	19.1062	95.5311
2	30	1.2222	29.8372	99.4572
3	40	1.6495	40.4957	101.2394
4	50	2.0822	51.2890	102.5781
5	60	2.4392	60.1941	100.3234
6	75	3.0022	74.2376	98.9837
7	100	4.0286	99.8401	99.8402
			Mean	99.7075
			S.D.	2.1990
			C.V.[c]	2.2054

a : obtained from the fit curve:

Peak Area Ratio = 0.0260 + (0.0401 x concentration); r^2 = 0.999

$$\text{Inversely Estimated Concentration} = \frac{\text{Peak Area Ratio} - 0.0260}{0.0401}$$

b : $$\%\ \text{Theory} = \frac{\text{Inversely Estimated Concentration} \times 100}{\text{Known Concentration}}$$

c : $$\text{Coefficient of Variation} = \frac{\text{SD} \times 100}{\text{Mean}}$$

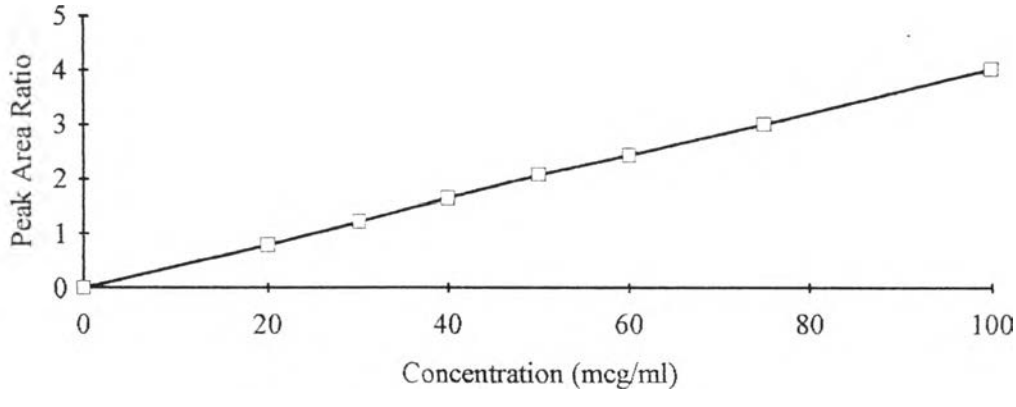

Figure 8. A Calibration Curve of Clindamycin Hydrochloride Solution.

Stability Study of Clindamycin Hydrochloride Gel.

Physical Stability Study.

Physical stability of all formulations had been studied before and after Freeze-Thaw cycles. The three gel preparations were clear, consistent, colorless, odorless and free from air bubble before and after eight Freeze-Thaw cycles. They were applied easily to the skin without residue.

The pH of the three formulations were measured before and after Freeze-Thaw cycles. The data of the pH values were shown in Table 6. The pH values of all formulations were in the range of pH-requirement (4.5 ± 0.2) after fresh preparing even after passing eight Freeze-Thaw cycles.

The data of viscosity of the three formulations before and after Freeze-Thaw cycles were exhibited in Table 7. The results showed a drop in viscosity after passing Freeze-Thaw cycles. The percentage of decrease in viscosity of Formulation I, II, III were 24.43, 11.66, and 7.31, respectively. Physically bonded gel networks are reversible system; factors such as temperature can induce a transition between the sol and gel phase. Shifts in temperature may cause an elastic concentration of polymer chains, which result in a drop in viscosity of cellulose derivatives gel (Swarbrick, 1988). In the case of thermoreversible gel, poloxamer 407, which became gel with heating and melted with cooling. The variation of temperature may cause in permanent viscosity change. The viscometer used in this study was a T-bar type spindle model, which could not control temperature during operating. This may be the reason of extreme drop in poloxamer 407 gel viscosity.

Table 6. The pH Values of Clindamycin Hydrochloride Gel before and after Eight Freeze-Thaw Cycles.

Formulation[a]	pH Value	
	Before Freeze-Thaw[b]	After Freeze-Thaw[b]
I	4.61 ± 0.011	4.50 ± 0.015
II	4.47 ± 0.011	4.37 ± 0.005
III	4.49 ± 0.005	4.40 ± 0.017

a : Formulation I = poloxamer 407 as gelling agent
Formulation II = hydroxyethyl cellulose as gelling agent
Formulation III = hydroxypropyl methylcellulose as gelling agent

b : Mean ± SD, (n = 3)

Table 7. The Viscosity Values (cps) of Clindamycin Hydrochloride Gel before and after Eight Freeze-Thaw Cycles.

Formulation	Viscosity Values	
	Before Freeze-Thaw[a]	After Freeze-Thaw[a]
I	3267 ± 55.9406	2469 ± 102.0016
II	1192 ± 11.0604	1053 ± 9.2376
III	219 ± 4.3589	203 ± 6.1441

a: Mean ± SD, (n = 3)

Chemical Stability Study.

The chemical stability of clindamycin hydrochloride gels kept at Joel-Davis condition and ambient temperature had been studied for about four months. The amount of drug before experimental testing presented as percent labelld amount was shown in Table 8. They were in the officially acceptable range of 90-110 % (Linter, 1973). The rate constant obtained from the concentration-time profile (Figure 9, 10) and ln(concentration)-time profile (Figure 11, 12) of the three gel formulations at ambient temperature and Joel-Davis condition were shown in Table 9 and 10, respectively. Since the slope are close to zero, their significance are tested using the t test with the null hypothesis H_0: B = 0 versus the alternative hypothesis H_a : B ≠ 0, where B is the slope values. The t statistics are obtained from:

$$t_{(d.f.,\ 0.975)} = \frac{b}{S_b}$$

where b is a sample estimate of true slope, B, and S_b is its variance. The value of t statistics were refered to the t distribution with (N-2) degree of freedom at the significance level of 0.05. In the case of ambient temperature the t statistics of all formulations in Table 9 were less than the $t_{(16,0.975)} = 2.120$. That is the null hypothesis H_0 : B = 0 is accepted, the slope was zero. From the statistical point of view all clindamycin hydrochloride gel preparations did not degrade chemically.

The same results were also obtained from the Joel-Davis tested condition. The t statistics of all formulations in Table 10 were less than $t_{(22,0.975)} = 2.074$. This also indicated that no chemical degradation occured during four months. According to the result obtained from this condition, it might conclude that all formulations of clindamycin hydrochloride gels had a two-year expiration peroid.

Table 11 showed that the percent remaining of drug in the Formulation I, II and III were 93.64 %, 98.38 % and 93.74 %, respectively after passing eight Freeze-Thaw cycles. Although, the percent remaining of drug in all formulations were higher than 90 %, the results from this condition did not

Table 8. Amounts of Clindamycin Hydrochloride and Percent Labelled Amount (% LA) of Clindamycin Hydrochloride in Three Gel Bases.

Formulation	Amounts of Clindamycin Hydrochloride[a] (mg/g)	% LA[a]
I	9.892 ± 0.44	98.922 ± 4.45
II	9.881 ± 0.28	98.807 ± 2.81
III	10.610 ± 0.05	106.102 ± 0.59

a : mean ± SD, (n=3)

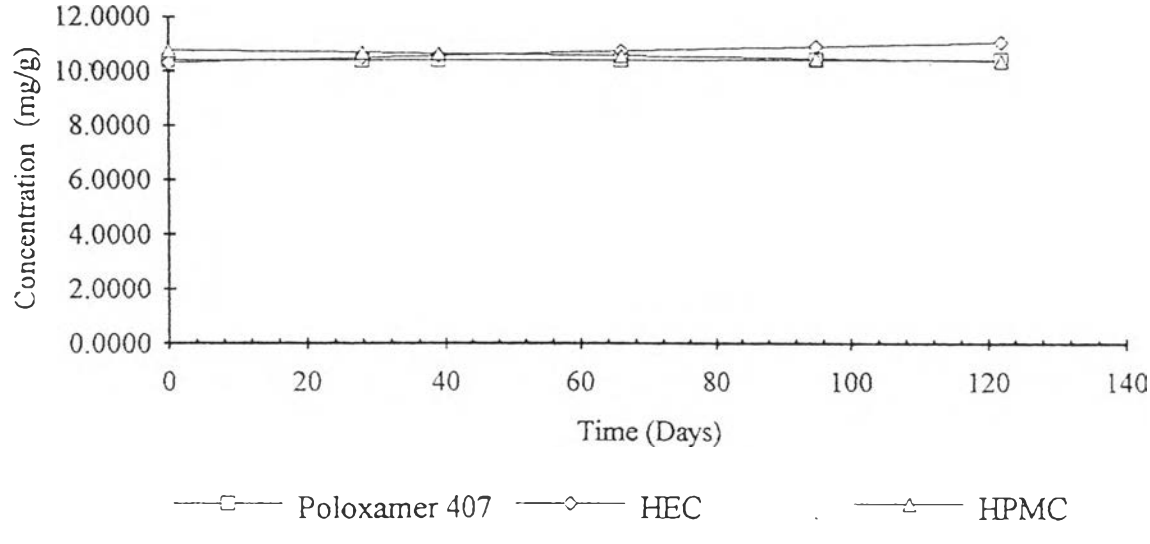

Figure 9. The Concentration-Time Profile of Clindamycin Hydrochloride Gels at Ambient Temperature.

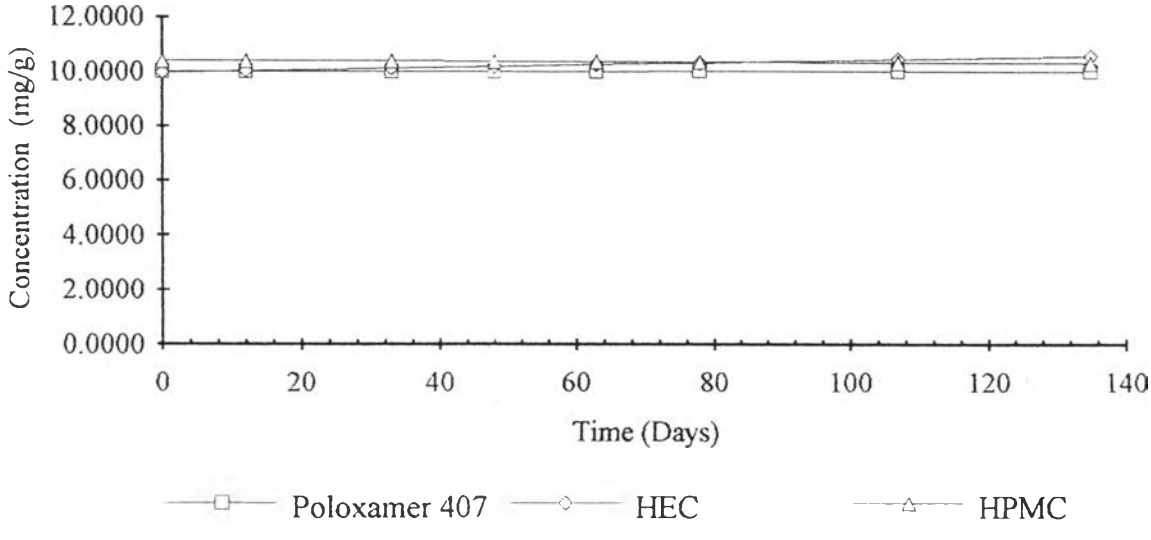

Figure 10. The Concentration - Time Profile of Clindamycin Hydrochloride Gels at Joel-Davis Condition (40°C, 80% RH.).

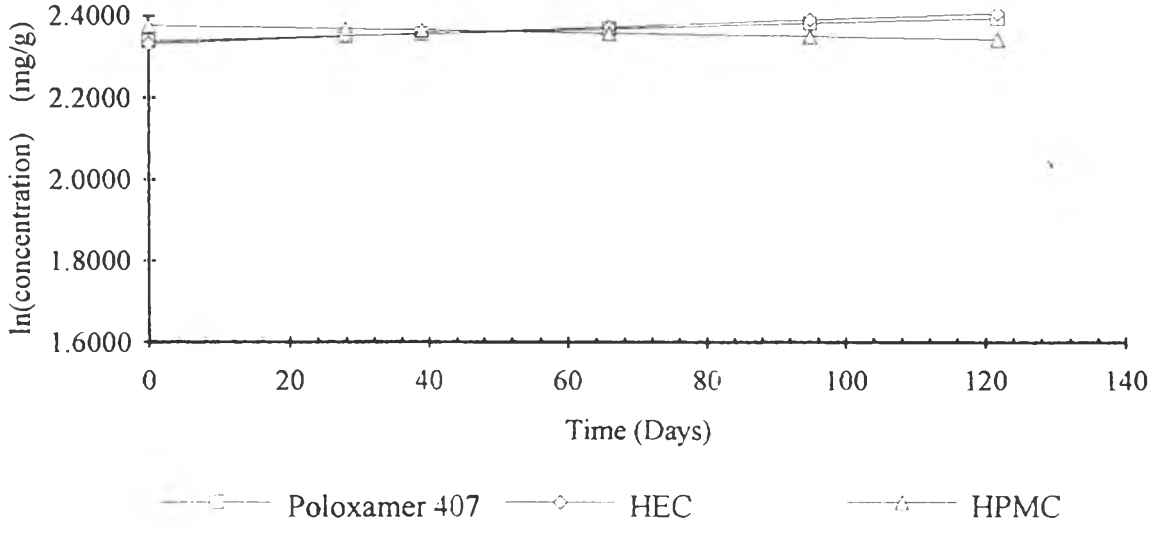

Figure 11. The ln(concentration)-Time Profile of Clindamycin Hydrochloride Gels at Ambient Temperature.

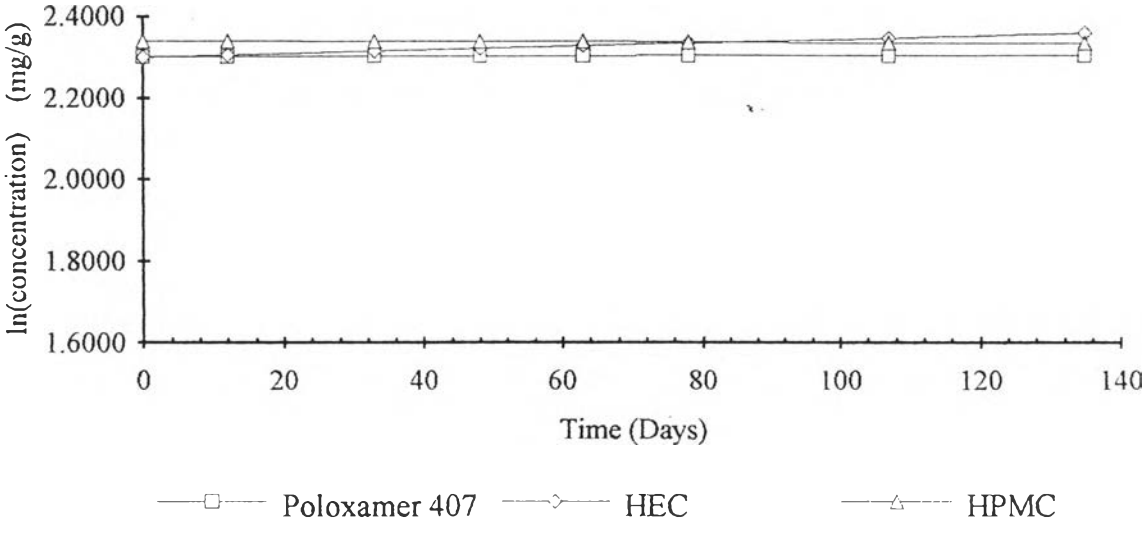

Figure 12. The ln(concentration)-Time Profile of Clindamycin Hydrochloride Gels at Joel-Davis Condition (40°C, 80% RH.).

Table 9. The Rate Constants and their t Statistics of the Prepared Clindamycin Hydrochloride Gel at Ambient Temperature.

Formulation	Zero order		First order	
	k_o[a] x 10^3 ($mg.day^{-1}$)	t	k_1[b] x 10^4 (day^{-1})	t
I	4.6231 ± 0.2352[c]	1.9660	4.6742 ± 2.9303[c]	1.5951
II	6.4422 ± 4.3667	1.4753	6.0830 ± 4.1604	1.4621
III	2.8814 ± 1.5245	1.8901	2.7185 ± 1.4532	1.8707

a : k_o was obtained from the slope of concentration vs time curve (zero-order degradation).

b : k_1 was obtained from the slope of ln (concentration) vs time curve (first-order degradation).

c : the variance of the estimate of the rate constant.

Table 10. The Rate Constants and their t Statistics of the Prepared Clindamycin Hydrochloride Gel at Joel-Davis Condition (40°C, 80% RH).

Formulation	Zero order		First order	
	k_o[a] x 10^3 (mg.day^{-1})	t	k_1[b] x 10^4 (day^{-1})	t
I	0.2590 ± 0.2543[c]	1.0185	0.2898 ± 0.2640[c]	1.0976
II	4.5382 ± 2.4192	1.8759	4.4219 ± 2.3528	1.8794
III	0.3482 ± 0.4131	0.8428	0.3680 ± 0.3537	1.0403

a : k_o was obtained from the slope of concentration vs time curve (zero-order degradation).

b : k_1 was obtained from the slope of ln (concentration) vs time curve (first-order degradation).

c : the variance of the estimate of the rate constant.

Table 11. Percent Remaining of Clindamycin Hydrochloride in the Gel Preparations after Passing Eight Freeze-Thaw Cycles.

Formulation	Percent Remaining[a]
I	93.64 ± 1.05
II	98.38 ± 2.24
III	93.74 ± 0.74

a : Mean ± SD, (n = 3)

correlate to those from other conditions. This indicated that the Freeze-Thaw condition was not suitable for the performance of chemical stability test.

The good chemical stability of clindamycin hydrochloride gels obtained from different storage condition might be the result of the following effects.

a. The pH of the formulations could maintain at maximum stability of drug over the period of experiment (Collett, 1990).

b. According to the gel structure, the net work limits fluid flow by entrapment and immobilization of the solvent molecule (Yalkawsky, 1979), which may cause a difficulty of drug and water interaction.

It was expected earlier that the buffered gel base would prevent the degradation of clindamycin hydrochloride. The results from this study supported that hypothesis.

Release Characteristics of Clindamycin Hydrochloride from Gel Preparations.

A method used to assess the release of drug from a preparation involved the diffusion of drug through a membrane to a receiving medium. The receiving solution chosen should be similar to the biological fluid and the drug should be dissolved completely in the system.

Clindamycin hydrochloride is very soluble in an aqueous solution but partially soluble or insoluble in a lipophilic solvent. However, choosing the aqueous receiving solution for testing the release characteristics of a gel preparation may cause a back diffusion to the donor compartment. An attempt to obtain a suitable receiving solution was made by considering two types of receiving media that were acetate buffer which represented the aqueous system and chloroform to represent the nonaqueous system.

Durapore(R), Fluoropore(R), and Nylon 66 membranes were studied as prototypes of hydrophilic, hydrophobic, and dialysis membranes, respectively. To study the release characteristics of a drug, the membranes used should not be

a barrier for diffusion but it should act as a border between the donor and receiving compartments. The three kinds of membranes were used for proving this assumption.

The performance of clindamycin hydrochloride gel preparations was evaluated by the *in vitro* release characteristics. The quantitation of drug releasing from the preparation was determined using the same method of analysis as in the case of the quantitation of drug in preparations which was previously described. All of the drug release data from the three gel formulations were exhibited in Appendix IV. The cumulative amount of drug release were plotted against square root of time and also against time. The release rate profiles of clindamycin hydrochloride are also shown in Appendix IV. If the release of drug was controlled by the diffusion of drug through the gel matrix, the cumulative amount release vs square root of time should be more correlated than the cumulative amount release vs time. In this case, the use of equation: $M = 2C_0A\sqrt{Dt/\pi}$ is valid within 30% cumulative amount release. On the other hand, if the drug release was membrane controlled, the cumulative amount release vs time should be more correlated since the equation: $M = C_dAPt$ would be valid. Table 12 shows the coefficient of determination of the plots using acetate buffer as a receiving solution. Table 13 shows those using chloroform as a receiving solution. However, the conclusion could not be made by considering the r^2 values.

Comparison of Drug Release from Gel Preparations.

If the release of drug was gel matrix-controlled, the Higuchi's equation ($M = 2C_0A\sqrt{Dt/\pi}$) would be valid. Using the Higuchi's equation, the diffusion coefficients (D) of clindamycin hydrochloride from different bases were calculated from the slope of cumulative amount release vs square root of time ($D = (slope/2C_0A)^2 \pi$) and were shown in Table 14, 15. From the diffusion coefficient data, the hydroxyethyl cellulose base yielded the lowest diffusion coefficient values in both receiving media and all membrane types, whereas the poloxamer 407 base gave the highest values in all cases. This informed the offhand information that the membranes and receiving solutions did not influence the comparison of drug release and it did confirm the matrix-controlled of drug release.

Table 12. The Coefficient of Determination (r^2) of the Plots of Amount Release (M) versus Square Root of Time ($t^{1/2}$) and versus Time (t) Using Acetate Buffer as a Receiving Solution.

Formulation	r^2 a					
	Durapore (R)		Fluoropore (R)		Nylon 66	
	M vs $t^{1/2}$	M vs t	M vs $t^{1/2}$	M vs t	M vs $t^{1/2}$	M vs t
I	0.9741±0.0028	0.9998±0.0001	0.9791±0.0036	0.9960±0.0035	0.9747±0.0030	0.9996±0.0001
II	0.9940±0.0048	0.9878±0.0052	0.9965±1.0011	0.9872±0.0019	0.9917±0.0012	0.9920±0.0023
III	0.9812±0.0013	0.9985±0.0056	0.9789±0.0045	0.9984±0.0013	0.9721±0.0037	0.9994±0.0038

a: Mean ± SD, (n =3)

Table 13. The Coefficient of Determination (r^2) of the Plots of Amount Release (M) versus Square Root of Time ($t^{1/2}$) and versus Time (t) Using Chloroform as a Receiving Solution.

Formulation	r^2 a					
	Durapore (R)		Fluoropore (R)		Nylon 66	
	M vs $t^{1/2}$	M vs t	M vs $t^{1/2}$	M vs t	M vs $t^{1/2}$	M vs t
I	0.9705±0.0065	0.9993±0.0004	0.9947±0.0060	0.9813±0.0022	0.9775±0.0044	0.9983±0.0007
II	0.9992±0.0006	0.9687±0.0060	0.9970±0.0012	0.9863±0.0041	0.9997±0.0002	0.9692±0.0065
III	0.9956±0.0022	0.9879±0.0036	0.9969±0.0017	0.9859±0.0034	0.9974±0.0015	0.9841±0.0044

a: Mean ± SD, (n =3)

Table 14. Diffusion Coefficients (D) Obtained in Acetate Buffer.

Formulation	Diffusion Coefficients[a] (D) x 10^4 cm^2/min		
	Durapore(R)	Fluoropore(R)	Nylon 66
I	8.4513 ± 0.1513	15.8513 ± 0.5075	14.8298 ± 0.5281
II	1.8865 ± 0.2811	4.7280 ± 0.2401	5.9811 ± 0.2840
III	4.1846 ± 0.9649	8.8533 ± 0.5344	11.7040 ± 0.4485

a : Mean ± SD, (n = 3)

Table 15. Diffusion Coefficients (D) Obtained in Chloroform.

Formulation	Diffusion Coefficients[a] (D) x 10^4 cm^2/min		
	Durapore(R)	Fluoropore(R)	Nylon 66
I	1.9217 ± 0.0584	2.1116 ± 0 1174	2.3107 ± 0.1757
II	0.4569 ± 0.0636	0.7575 ± 0 0244	1.3713 ± 0.0933
III	0.6657 ± 0.0156	0.8356 ± 0 0071	1.4735 ± 0.0545

a : Mean ± SD, (n = 3)

The gelling agent can modify the observed diffusivity of a solute by either mechanically impeding its movement or by adsorbing the solute on the polymer surface. Since the actual pathway for diffusion in gels is through the fluid phase, the factors which affect diffusivity in pure liquid phase are similar to the factors which control the diffusion within gels (Barry, 1983). With this assumption, the diffusivity should be releated to the frictional resistance (f) experienced by the diffusing particles, the gas constant (R), the absolute temperature (T), and the Avogadro's number (N) by the following equation (Jost, 1960) :

$$D = \frac{RT}{fN} \qquad \text{.....(eq 17)}$$

When a particle diffuses through a homogeneous liquid and the particles are large compared with the solvent molecules, equation 17 approaches the Stoke's expression :

$$D = \frac{RT}{6\eta\pi rN} \qquad \text{.....(eq 18)}$$

Here η is the vehicle viscosity and r is the hydrodynamic radius of the diffusing particles.

From equation 18, with other parameters being constant, the diffusion coefficient (D) is inversely proportional to the viscosity of the vehicle. The decrement in viscosity of the vehicle would increase the diffusivity of the drug and thus, increase the release rate. As far as the viscosity is concerned, the diffusion coefficient of clindamycin hydrochloride in poloxamer gel should be the lowest because of the highest viscosity of Formulation I as presented in Table 16. On the contary, the highest diffusivity should be obtained from the hydroxypropyl methylcellulose base because of its lowest viscosity. However, the calculated diffusion coefficient exhibited that the most viscous gel, poloxamer 407, presented the highest diffusivity. This may be due to the diffusion of salt across the membrane to the receiving media during the experiment. Examples of the salt are an acetate ion (from acetate buffer) and a hydrochloride which ionized from the drug. This would cause the decrease in

Table 16. Viscosities of the Clindamycin Hydrochloride Gel Preparations.

Formulation	Gelling agent	Concentration (% w/w)	Viscosity[a] (cps)
I	Poloxamer 407	18	3267 ± 55.9406
II	HEC	2	1192 ± 11.0604
III	HPMC	3	219 ± 4.3589

a: Mean ± SD, (n=3)

viscosity of the gel in the donor compartment and this was also observed during the run. Generally, a salt may be added to the poloxamer gel to increase its viscosity (Gilbert, et al., 1986).

The other reason for dropping in the gel viscosity is the diffusion of poloxamer 407 to the receiving solution. This was noticed by the appearance of air bubbles in the sampling solution which should be due to the surfactant property of poloxamer (Swarbrick,1988). A minimum concentration of poloxamer 407 to be able to form a gel structure in this system was 18% w/w. Any diffusion of poloxamer 407 to the receiving solution would make a more fluid donor gel. In conclusion, the viscosity of poloxamer 407 gel did not really represent its viscosity during the release experiments. The drop in viscosity during the run made the drug move faster and thus increased the diffusion coefficient of drug more than expected.

In the case of hydroxyethyl cellulose base, the lowest diffusion coefficient values may be attributed to the rigid gel structure of hydroxyethyl cellulose and the gel is jelly-liked with a high cross-link density (Swarbrick,1988).

According to the lowest viscosity and the liquid-gel appearance of hydroxypropyl methylcellulose base, the diffusion coefficient value of the drug in this base was greater than that obtained from hydroxyethyl cellulose.

Effect of the Polarity of Receiving Solutions on the Drug Release.

When the diffusion coefficients of drug using different receiving solutions but the same membrane and formulation were compared (Table 14 and 15), using chloroform as the receiving solution gave the lower D values than the case of acetate buffer. This should be attributed to the less affinity of drug to chloroform than to acetate buffer. Clindamycin used in this study was a hydrochloride salt so it is very soluble in acetate buffer. The drug solubility in chloroform is less than 1 mg/ml (Florey,1981). Therefore, a greater amount of drug remained in the gel base in the case of chloroform than that in the case of acetate buffer.

Although the absolute diffusion coefficients are different significantly in the two receiving solutions, both solutions can be used for comparison of drug release from gel preparations since the rank order of D's are the same.

Effect of the Membrane Types on the Drug Release.

Three different kinds of synthetic membranes were used in this study. A hydrophilic membrane (Durapore(R)) allowed the penetrating agents penetrate through the tortuous pores formed by the overlapping strands of polymer (Martin, Swarbrick and Cammarata,1983). Fluoropore(R) was a representative of a hydrophobic membrane. This membrane was completely repellent by aqueous solutions unless it was prewetted with methanol. Since Nylon 66 membrane is impermeable to small molecules such as water but is permeable to high molecular weight and unionized species, it was expected to solve the problem of back diffusion of the receiving medium.

The general rank order of clindamycin hydrochloride release through all types of membranes considering the diffusion coefficient as the release parameter was : poloxamer 407 > hydroxypropyl methylcellulose > hydroxyethyl cellulose (Table 14, 15). This indicated that any pore membranes did not influence the assessment of relative ability of drug release. There was no evidence that the receiving medium diffused back through the membranes during the experimental run.

The physicochemical properties of membrane were responsible for the differences in the D values when the release through different kinds of membranes were compared within the same formulation and receiving solution. Durapore(R) yielded the lowest D values since its pore diameter was smallest (0.45 micron) and its porosity was just 75%. Except from the case of Formulation I in acetate buffer, Fluoropore(R) gave the D values that were in between the Durapore(R) and Nylon 66 cases. This was because the pore diameter (0.5 micron) and % porosity (85%) of Fluoropore(R) were greater than those of Durapore(R). The pore diameters of Nylon 66 and Fluoropore(R) were the same. Therefore, the dialysis characteristics of Nylon 66 should allow the drug to be more permeable than through Fluoropore(R). Still, the D values of drug release from Formulation I through Fluoropore(R) (15.8513×10^{-4} cm^2/min) and

through Nylon 66 (14.8298×10^{-4} cm^2/min) to acetate buffer are comparable using the t test with the null hypothesis H_o: $D_{Fluoropore^{(R)}} - D_{Nylon\ 66} = 0$ versus the alternative hypothesis H_a: $D_{Fluoropore^{(R)}} - D_{Nylon\ 66} \neq 0$. The t statistics is obtained from:

$$t_{(df.,0.975)} = \frac{(D_{Fluoropore^{(R)}} - D_{Nylon\ 66})}{S_p^2/n_1 + S_p^2/n_2}$$

$$= 2.4160$$

where S_p^2 is pooled variance which is obtained from:

$$S_p^2 = \frac{(n_1 - 1)\, S_1^2 + (n_2 - 1)\, S_2^2}{(n_1 - 1) + (n_2 - 1)}$$

The value of t statistics was refered to the t distribution with $(n_1 - 1) + (n_2 - 1)$ degree of freedom at the significance level of 0.05. The calculated t value was less than the $t_{(4.,0.975)} = 2.776$. Therefore, the null hypothesis is accepted. Consequently, any pore membranes studied could be used for the comparison of drug release from different formulations.