CHAPTER IV

RESULTS AND DISCUSSION

1. Solubility of KP in various vehicles.

The solubility of KP in various vehicles was studied. It was found the solubility of KP in DI water was lower than in 5%w/w PG in DI water and 0.5%w/w TW80 but higher than in 1%w/w L-LA. Although the solubility of KP was increased when added to 5%w/w PG or 0.5%w/w TW80 in DI water but the solubility of KP in all vehicles were very slightly soluble according to the USP XXIII definition (34). The solubility of KP in various vehicles and pH of each vehicle are shown in Table 5.

Table 5 Solubility of KP in various vehicles

Vehicles	pH	Solubility (mg of solute/ml of solvent)		
		Mean	±	SD
DI water	6.52	0.137	±	0.01
5%w/w PG in DI water	5.65	0.505	±	0.02
0.5%w/w TW80 in DI water	5.54	0.218	±	0.02
1%w/w L-LA in DI water	1.95	0.115	±	0.01

2. Evaluation of KP gels.

The drug content, pH, and viscosity of all prepared gels were measured in triplicate and the average value expressed as mean±SD.

2.1 Drug content

The theoretical KP content was 2.50 %w/w and the average of actual KP content in all KP gels in this study was in the range of 2.34 to 2.66 %w/w. The average of KP content (%w/w) for each formulation is shown in Table 6.

Table 6 Actual amount of KP in KP gels

No.	Factor studies	Actual KP content (%w/w)
		Mean±SD
F-1	CBP2020 3%	2.50±0.02
F-2	CBP2020 2%	2.34±0.03
F-3	CBP2020 1.5%	2.43±0.09
F-4	CBP980 3%	2.55±0.18
F-5	CBP980 2%	2.65±0.06
F-6	CBP980 1.5%	2.49±0.14
F-7	HPMC 3%	2.45±0.08
F-8	HPMC 2% ETOH 35.5%	2.35±0.08
F-9	HPMC 2% ETOH 30%	2.43±0.21
F-10	HPMC 2% ETOH 40%	2.41±0.05
F-11	HPMC 2.5% pH 3.4	2.63±0.11
F-12	HPMC 2.5% pH 5.7	2.41±0.24
F-13	HPMC 2.5% pH 7.0	2.48±0.06
F-14	HPMC 3% Non-additive	2.43±0.07
F-15	HPMC 3% PG	2.41±0.06
F-16	HPMC 3% TW80	2.53±0.15
F-17	HPMC 3% L-LA	2.54±0.12
F-18	HPMC 3% PG-TW80	2.44±0.04
F-19	HPMC 3% PG-L-LA	2.56±0.06
F-20	HPMC 3% PG-TW80-L-LA	2.48±0.05
Commercial KP gel1		2.66±0.05
Commercial KP gel2		2.45±0.10

2.2 pH and viscosity of KP gels

The average pH of all gels in this experiment was in the range of 5.23 to 7.34 except the F-11, which was intended to study the effect of pH on *in vitro* permeation with a pH of 3.36. The viscosity of each formulation was depended on their compositions and the concentration of gelling agent used. The average pH and viscosity of all KP gels are shown in Table 7.

Table 7 Average pH and viscosity of KP gels

No.	Factor studies	pH	Viscosity (cP)
		Mean±SD	Mean±SD
F-1	CBP2020 3%	5.41 ± 0.01	16980 ±500
F-2	CBP2020 2%	5.36 ± 0.03	12413±390
F-3	CBP2020 1.5%	5.79 ± 0.01	9720±35
F-4	CBP980 3%	6.48 ± 0.01	19120±695
F-5	CBP980 2%	6.15 ± 0.01	15953±12
F-6	CBP980 1.5%	6.30 ± 0.01	10027±284
F-7	HPMC 3%	7.13 ± 0.01	12460±365
F-8	HPMC 2% ETOH 35.5%	5.62 ± 0.08	3647±50
F-9	HPMC 2% ETOH 30%	6.38 ± 0.02	3707±155
F-10	HPMC 2% ETOH 40%	6.09 ± 0.03	3747±12
F-11	HPMC 2.5% pH 3.4	3.36 ± 0.03	6507±70
F-12	HPMC 2.5% pH 5.7	5.67 ± 0.02	6573±12
F-13	HPMC 2.5% pH 7.0	6.97 ± 0.03	6273±81
F-14	HPMC 3% Non-additive	5.65 ± 0.06	13173±153
F-15	HPMC 3% PG	7.34 ± 0.02	14720±139
F-16	HPMC 3% TW80	6.74 ± 0.01	15360±164
F-17	HPMC 3% L-LA	5.38 ± 0.03	15447±42
F-18	HPMC 3% PG-TW80	5.75 ± 0.02	16040±72
F-19	HPMC 3% PG-L-LA	5.49 ± 0.07	14707±95
F-20	HPMC 3% PG-TW80-L-LA	5.23 ± 0.02	12960±20
Commercial KP gel1		5.76 ± 0.02	5867±50
Commercial KP gel2		6.55 ± 0.03	10787±99

3 *In vitro* permeation of KP

In vitro permeation of KP from KP gels was studied using full-thickness Wistar rat skin as a model membrane. The mean cumulative amount of KP permeated at each time interval from 10 to 180 min was calculated. The data was plotted between cumulative KP permeated ($\mu g/cm^2$) and time (min). The slope at steady state (flux) and cumulative amount of KP permeated at the end of the experiment at 180 min (Q_{180min}) were obtained from each plot and used as indicators to compare between formulations. Both parameters were statistically tested by one-way analysis of variances and the least square difference methods, at the 95% of confidence level.

3.1 Effects of gelling agents on KP permeation flux and Q_{180min}

The three types of gelling agents used in this study were CBP2020, CBP980, and HPMC. The 3% w/w of these gelling agents were used to formulate the gel F-1, F-4, and F-7. The permeation profiles show in Table 8 and Figure 5. Table 8 compares the cumulative amount of KP permeated at each sampling time and the permeation parameters. The fluxes of F-1, F-4, and F-7 were 0.054, 0.040, and 0.044 $\mu g/cm^2$ /min and the Q_{180min} were 6.710, 4.890, and 5.550 $\mu g/cm^2$, respectively. Between the three formulations, both the flux and Q_{180min} were not significantly different. The same results were observed from the gel containing 2 %w/w gelling agents, F-2, F-5, and F-8. At the lower concentration, 1.5% w/w, of CBP2020 and CBP980 (F-3 and F-6), it was found the KP permeated from CBP2020 was higher than CBP980. The effects of gelling agent types on KP *in vitro* permeation was not significantly different when using 2% w/w or 3% w/w of gelling agent. This may be due to similar properties of all gelling agents used in this study. Although, CBP2020 and CBP980 are ionic hydrophilic polymers and HPMC is a nonionic hydrophilic polymer, all formulations were adjusted to the similar pH that may minimize the ionization properties of the gelling agents.

Table 8 Effects of gelling agents on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)		
	F-1 (CBP2020)	F-4 (CBP980)	F-7 (HPMC)
	Mean ± SD	Mean ± SD	Mean ± SD
10	0 ± 0	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0	0 ± 0
30	0 ± 0	0 ± 0	0 ± 0
60	0 ± 0	0 ± 0	0 ± 0
90	2.452 ± 1.30	1.626 ± 0.90	2.034 ± 0.149
120	3.913 ± 1.02	2.907 ± 0.66	2.725 ± 0.065
180	6.707 ± 0.97	4.885 ± 1.18	4.265 ± 0.79
Flux ($\mu g/cm^2/min$)	0.054 ± 0.01	0.040 ± 0.01	0.044 ± 0.00
$Q_{180\ min}$ ($\mu g/cm^2$)	6.707 ± 0.97	4.885 ± 1.18	4.265 ± 0.79

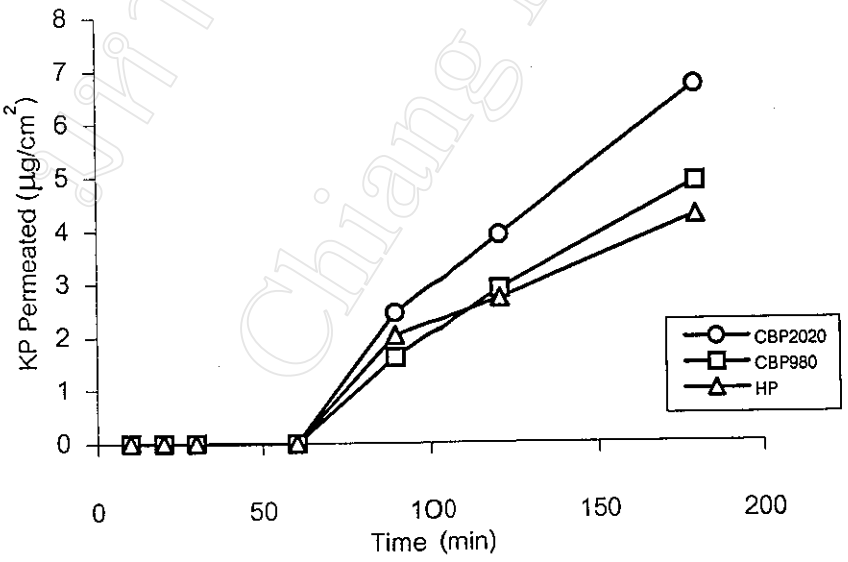

Figure 5 Effects of gelling agents on KP permeation through rat skin

3.2 Effects of gelling agent concentrations on KP permeation flux and Q_{180min}

The three concentrations, 60, 40, and 30 g, of 5%w/w hydrated stock gel used in the formulation correspond with 3, 2, and 1.5%w/w, respectively, of dry CBP2020 and CBP980. In the case of HPMC only 60 and 40 g of 5%w/w hydrated stock gel were used to prepare proper viscosity of gels.

3.2.1 Effects of CBP2020 Concentrations on KP permeation flux and Q_{180min}

F-1, F-2, and F-3 were prepared with CBP2020 3%, 2% and 1.5%w/w, respectively. The mean cumulative amount of KP permeated at each sampling time and the permeation parameters are shown in Table 9 and Figure 6. Although the concentration of gelling agent was increased, the KP permeation flux and Q_{180min} was not significantly different.

Some investigators have suggested that the flux of KP permeated from oleohydrogels decreased exponentially as a function of Carbopol®940 (CBP940) concentration due to a reduction in the amount of released drug molecules available for the permeation through the skin (2). Similar results were observed in this study when CBP2020 of different concentrations were used. The fluxes of KP from 1.5%,2%, and 3%w/w CBP2020 gels were 0.066, 0.059, and 0.054 $\mu g/cm^2$ /min and Q_{180min} were 11.370, 10.620, and 6.710 $\mu g/cm^2$, respectively. However, these results were not significantly different. The higher concentration of gelling agent resulted in higher viscosity of the gels which vary from 9720, 12413, and 16980 cP for 1.5%, 2%, and 3%w/w of gelling agent used in the formulations, but the different concentrations of CBP2020 have no pronounced effect on *in vitro* permeation of KP gels. The lag time was significantly increased as gelling agent increase from 2% to 3%w/w.

Table 9 Effects of CBP2020 concentration on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)		
	F-1 (CBP2020 3%)	F-2 (CBP2020 2%)	F-3 (CBP2020 1.5%)
	Mean ± SD	Mean ± SD	Mean ± SD
10	0 ± 0	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0	0 ± 0
30	0 ± 0	2.457 ± 0.55	1.947 ± 0.08
60	0 ± 0	3.937 ± 1.34	2.989 ± 0.01
90	2.452 ± 1.30	4.75 ± 1.33	4.601 ± 0.56
120	3.913 ± 1.02	6.312 ± 1.74	6.341 ± 0.90
180	6.707 ± 0.97	10.622 ± 3.55	11.374 ± 1.94
Flux ($\mu g/cm^2/min$)	0.054 ± 0.01	0.059 ± 0.02	0.066 ± 0.01
$Q_{180\ min}$ ($\mu g/cm^2$)	6.707 ± 0.97	10.620 ± 3.55	11.374 ± 1.94

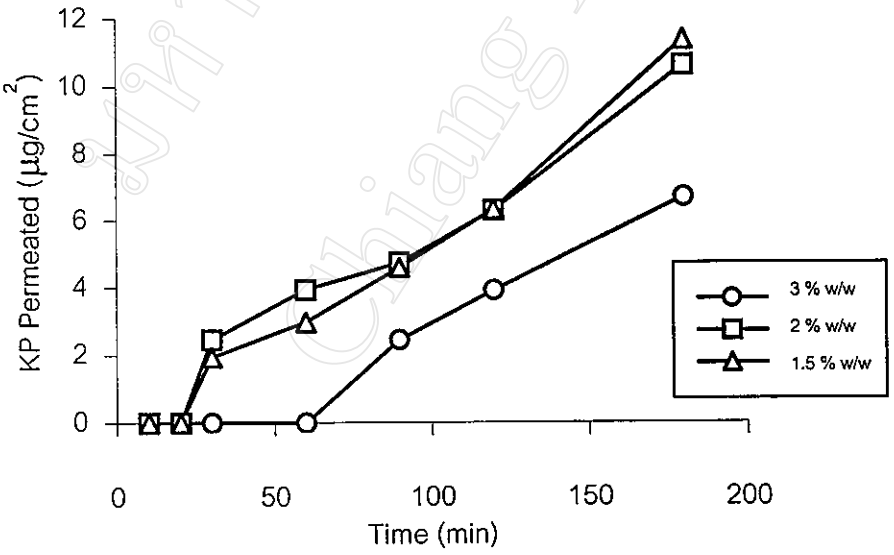

Figure 6 Effects of CBP2020 concentrations on KP permeation through rat skin

3.2.2 Effects of CBP980 concentrations on KP permeation flux and Q_{180min}

F-4, F-5, and F-6 were prepared with CBP980 3%, 2% and 1.5%w/w, respectively. Figure 7 and Table 10 show the means cumulative amount of KP permeated at each sampling time and the permeation parameters. The fluxes and Q_{180min} of these three concentrations of CBP980 were similar.

Three concentrations of CBP980 were used. It was found the higher concentration of gelling agent caused higher viscosity of the gel but similar permeation profiles and permeation parameters were observed. When the concentration of CBP980 was increased to 1.5%, 2%, and 3%w/w, the fluxes were 0.038, 0.043, and 0.040 $\mu g/cm^2$/min and the Q_{180min} were 4.86, 5.45, and 4.89 $\mu g/cm^2$. These results were similar and not significantly different. The different concentrations in the range from 1.5% to 3% w/w of CBP980 were not significantly different on KP permeation of these gels.

Table 10 Effects of CBP980 concentrations on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)		
	F-4 (CBP980 3%)	F-5 (CBP980 2%)	F-6 (CBP980 1.5%)
	Mean ± SD	Mean ± SD	Mean ± SD
10	0 ± 0	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0	0 ± 0
30	0 ± 0	0 ± 0	0 ± 0
60	0 ± 0	0 ± 0	0 ± 0
90	1.626 ± 0.90	2.479 ± 0.35	2.088 ± 0.19
120	2.907 ± 0.66	3.198 ± 0.48	2.540 ± 0.07
180	4.885 ± 1.18	5.449 ± 0.56	4.861 ± 0.59
Flux ($\mu g/cm^2/min$)	0.040 ± 0.01	0.043 ± 0.01	0.038 ± 0.01
$Q_{180\ min}$ ($\mu g/cm^2$)	4.885 ± 1.18	5.449 ± 0.56	4.861 ± 0.59

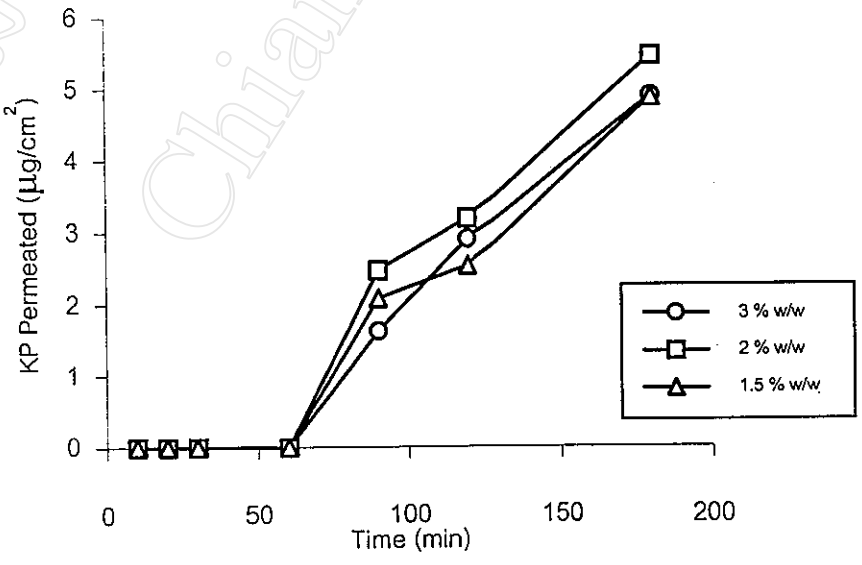

Figure 7 Effects of CBP980 concentrations on KP permeation through rat skin

3.2.3 Effects of HP concentrations on KP permeation flux and Q_{180min}

KP gel prepared with HP 3% and 2%w/w was used in F-7 and F-8. The mean cumulative amount of KP permeated at each sampling time and the permeation parameters show in Figure 8 and Table 11. The fluxes were slightly increased but the Q_{180min} was slightly decreased when the concentration of HP was increased. The KP fluxes of 2% and 3% HP gel were 0.038 and 0.044 $\mu g/cm^2$ /min while the Q_{180min} were 6.05 and 5.55 $\mu g/cm^2$. The KP permeation flux and Q_{180min} were not significantly different. Although, the 2% and 3%w/w of HP increased gel viscosity from 3647 to 12460 cP but there was no pronounced effect on the *in vitro* permeation of KP except the lag time was prolonged as HP was increased.

Table 11 Effects of HP concentrations on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)	
	F-7 (HP 3%)	F-8 (HP 2%)
	Mean ± SD	Mean ± SD
10	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0
30	0 ± 0	0 ± 0
60	0 ± 0	2.033 ± 0.46
90	2.034 ± 0.15	2.917 ± 0.27
120	2.725 ± 0.06	3.804 ± 0.31
180	4.265 ± 0.79	6.054 ± 1.74
Flux ($\mu g/cm^2/min$)	0.044 ± 0.00	0.038 ± 0.01
$Q_{180\ min}$ ($\mu g/cm^2$)	5.55 ± 0.37	6.05 ± 1.74

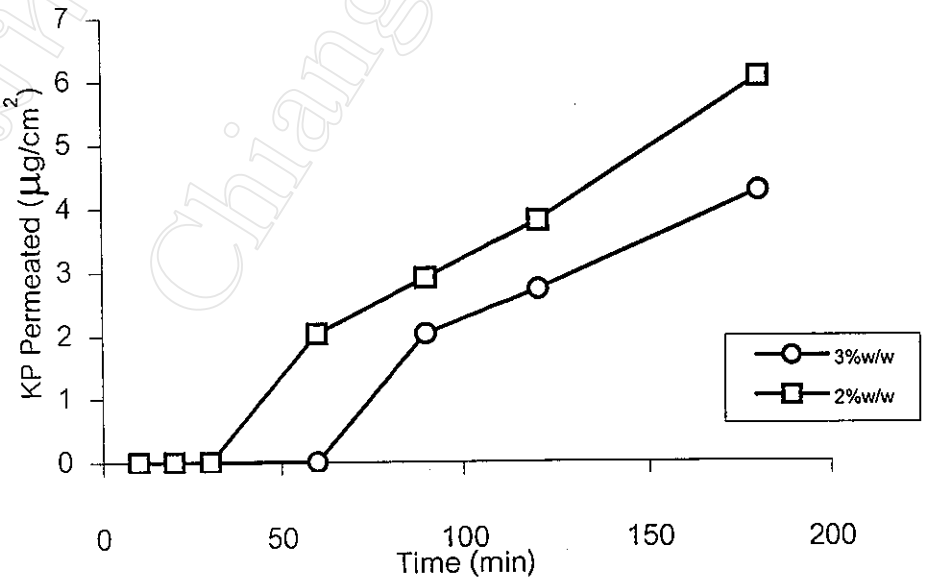

Figure 8 Effects of HP concentrations on KP permeation through rat skin

3.3 Effects of ethanol concentrations on KP permeation flux and Q_{180min}

Effects of the ETOH concentration on the cumulative amount of KP permeation profile and other parameters of F-9, F-8, and F-10 are shown in Table12 and Figure 9. The concentrations of ETOH in F-9, F-8, and F-10 were 30%, 35.5%, and 40% w/w. The permeation flux of F-9, F-8, and F-10 were 0.026, 0.038, and 0.033 $\mu g/cm^2$ /min, respectively. The Q_{180min} of these formulations were 5.944, 6.054, and 7.573 $\mu g/cm^2$, respectively. Both flux and Q_{180min} of these three formulations were not significantly different.

ETOH in this experiment was used as a solvent for KP which was freely soluble in ETOH. It also used as a preservative because of its high concentration (>20%w/w) in each formulation. ETOH also acts as an enhancer. Berner *et al.*, found that ETOH could increase the nitroglycerin (lipophilic drug) flux and the optimum volume fraction of ETOH to increase the flux of this drug across the skin was less than or equal to 0.7 (15). Kurihara-Bergstrom T., *et al.*, concluded that ETOH-water mixed systems enhance permeation of the salicylate ion (ionic molecule) through the stratum corneum. The optimum volume fraction of ETOH to increase the salicylic ion was nearly 0.63 (16). Eiichiro Manabe, *et al.*, reported that the combination of low concentration ETOH and water increased the skin penetration of isosorbide dinitrate and nitroglycerin but the flux could be decreased when a higher concentration of ETOH was used (18). The mechanism of ETOH to enhance the skin permeability of various drugs has been reported in some papers. For lipophilic drug, the absorption of ETOH and water is enhanced into the stratum corneum and disturbed the lipid part of the stratum corneum. The barrier function of the stratum corneum was reduced and the drug could more easily penetrate into the skin. At higher concentration of ETOH (more than 40%w/w) the skin was delipidized, thus, the barrier function of the stratum corneum to overall permeation of the drug was reduced (15). Yoichi Kobayashi, *et al.*, suggested that ETOH increased drug permeability through the entire region of the skin (19). Angela K

Levang, *et al.*, explained that the ETOH and PG solvent system increased the penetration of aspirin by perturbing the macroscopic barrier integrity of the stratum corneum and the stratum corneum lipids were lost. (14) For an ionic molecule, Kurihara-Bergstrom T., *et al.*, suggested the enhancement effects of ETOH may be due to the alteration of the polar pathway. This alteration may occur in either or both the lipid polar head and proteinaceous region of the stratum corneum and ion pairs formation. However, the permeation of the ion decreased at higher volume fractions of ETOH (more than 0.63) that because the uptake of permeant into the stratum corneum was decreased.

In this experiment, ETOH concentration was increased from 30%w/w to 40%w/w. The permeation flux and Q_{180min} of KP were not significantly different. In this study, ETOH concentration in the range of 30%w/w to 40%w/w had no pronounce effect on *in vitro* permeation of KP form KP gels made of HPLC as shown in Table 12 and Figure 9.

Table 12 Effects of ethanol concentrations on KP permeation flux and Q_{180min}

Time (min)	KP Permeated (µg/cm^2)		
	F-9 (ETOH 30%)	F-8 (ETOH 35.5%)	F-10 (ETOH 40%)
	Mean ± SD	Mean ± SD	Mean ± SD
10	0.807 ± 1.40	0 ± 0	0 ± 0
20	0.934 ± 1.62	0 ± 0	2.355 ± 0.18
30	1.232 ± 2.13	0 ± 0	2.770 ± 0.17
60	2.611 ± 2.34	2.033 ± 0.46	4.464 ± 0.65
90	4.207 ± 0.85	2.917 ± 0.27	5.054 ± 0.76
120	5.165 ± 0.45	3.804 ± 0.31	6.375 ± 0.58
180	5.944 ± 0.49	6.054 ± 1.74	7.573 ± 1.42
Flux (µg/cm^2/min)	0.026 ± 0.02	0.038 ± 0.01	0.033 ± 0.01
$Q_{180\ min}$ (µg/cm^2)	5.944 ± 0.49	6.054 ± 1.74	7.573 ± 1.42

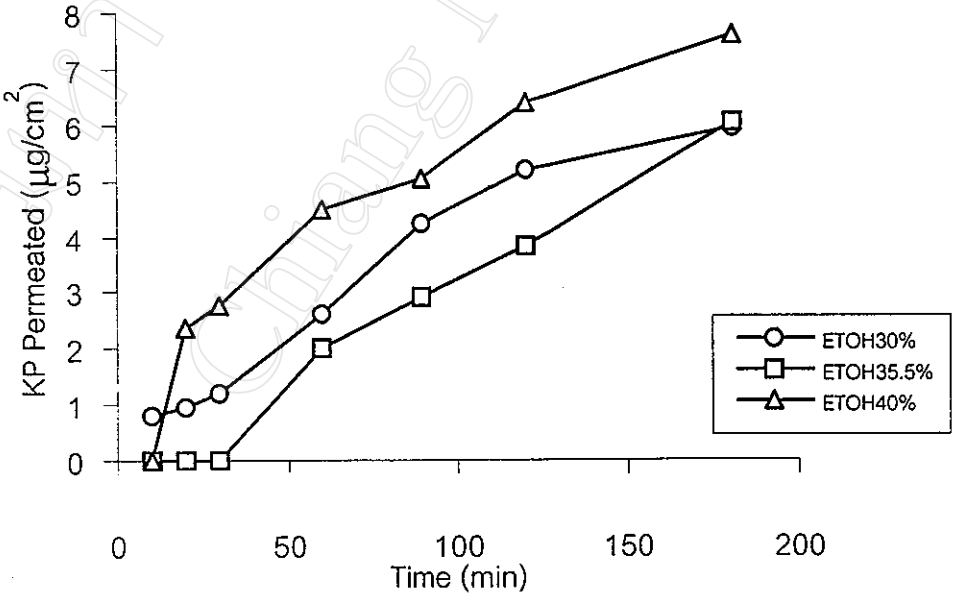

Figure 9 Effects of ETOH concentrations on KP permeation through rat skin

3.4 Effects of pH of formulation on KP permeation flux and Q_{180min}

Ketoprofen is a weak acid; pKa 4.45 (31) has strong lipophilic character, that is shown by the low solubility in water (34), the pH of gel would affect the overall permeation flux of KP. In this study, the effect of pH on permeation of KP was investigated over the pH 3.4 to 7.0 range. The pH of KP gel prepared with 3%w/w HP was adjusted to the desire pH of 3.4, 5.7, and 7.0 by adding a proper amount of triethanolamine, the gels obtained were assigned as F-11, F-12, and F-13, respectively.

The mean of cumulative amount of KP permeated at each sampling time and the permeation parameters are shown in Table 13. Figure 10 shows that KP fluxes are higher at lower pH than at higher pH, due to more un-ionized KP at pH 3.4 than at pH 5.7 and pH 7.0. This is consistent with the principle of non-ionic diffusion through a biological membrane. On the other hand, Gye Ju Rhee *et al.*, reported that, the pH dependence of KP oleo-hydrogel on the permeation of KP was not significant. This was due to the KP was in emulsion droplets containing an oil component, the diffusable un-ionized form of KP in the aqueous gel may not be responsible for the skin permeation of the drug (2).

Table 13 Effects of pH on KP permeation flux and Q_{180min}

Time (min)	KP Permeated (µg/cm^2)		
	F-11 (pH3.4)	F-12 (pH5.7)	F-13 (pH7.0)
	Mean ± SD	Mean ± SD	Mean ± SD
10	0.981 ± 1.70	0.832 ± 1.44	3.157 ± 0.25
20	1.181 ± 2.05	1.693 ± 1.49	3.553 ± 0.24
30	2.296 ± 2.10	2.000 ± 1.79	4.024 ± 0.22
60	4.398 ± 1.90	2.927 ± 0.74	4.580 ± 0.33
90	7.570 ± 1.77	4.000 ± 0.86	5.063 ± 0.44
120	11.302 ± 1.62	5.122 ± 0.99	5.761 ± 0.73
180	19.678 ± 4.34	7.535 ± 1.83	6.781 ± 0.95
Flux (µg/cm^2/min)	0.110 ± 0.02	0.037 ± 0.00	0.021 ± 0.01
$Q_{180\ min}$ (µg/cm^2)	19.678 ± 4.34	7.535 ± 1.83	6.781 ± 0.95

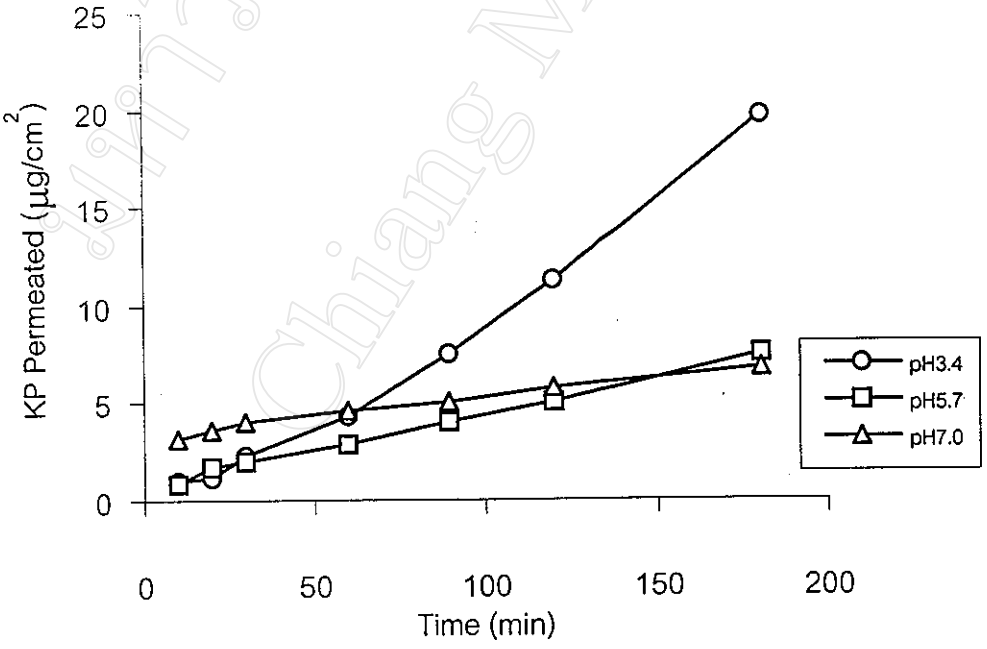

Figure 10 Effects of pH on KP permeation through rat skin

3.5 Effects of additives on KP permeation flux and Q_{180min}

3.5.1 Effects of mono-additives on KP permeation flux and Q_{180min}

The stratum corneum of the skin has a strong barrier function. Only a small number of drugs permeate the skin in therapeutic amount. The skin permeations are required in most topical preparations. The additives in this study were 5%w/w PG, 0.5%w/w TW80, and 1%w/w L-LA. All these substances were used as the penetration enhancers in the F-15, F-16, F-17. The mean of cumulative amount KP permeated at each sampling time and the permeation parameters are shown in Table 14. The permeation profiles are shown in Figure 11. The permeation flux and Q_{180min} of formulation with 1%w/w L-LA was significantly higher than formulation without additive, with 5%w/w PG or 0.5%w/w TW80.

PG is generally regarded as a non toxic material and widely use as a solvent, cosolvent and skin permeation enhancer. In this experiment, formulation with of 5%w/w PG did not increase the permeability of KP when compared with formulation without additive. The permeation flux of 5%w/w PG formulation was 0.031 $\mu g/cm^2$/min while the permeation flux of non-additive formulation was 0.059 $\mu g/cm^2$/min. The Q_{180min} of 5%w/w PG formulation was 4.89 $\mu g/cm^2$ while the Q_{180min} of non-additive formulation was 8.85 $\mu g/cm^2$. Barry B.W. suggested that the mechanism of PG to enhance skin permeability is effective when the stratum corneum is not fully hydrated. He explained that PG probably operates by solvating the keratin and occupying hydrogen-bonding sites, thus reducing drug/tissue binding. In some conditions when applied PG alone to fully hydrated tissue, it could not increase drug penetration (13).

The permeation flux and Q_{180min} of formulation containing 0.5%w/w TW80 were significantly lower than without additive formulation. The permeation flux of 0.5%w/w TW80 formulation was 0.029 $\mu g/cm^2$/min while the permeation flux of formulation without additive was 0.059 $\mu g/cm^2$/min. At the same time, Q_{180min} of 0.5%w/w TW80 formulation

was 2.94 μg/cm^2 while the Q_{180min} of formulation without additive was 8.85 μg/cm^2. The decrease of permeation flux and Q_{180min} may be due to KP solubility in 0.5%w/w TW80 was increased (Table 5) or TW80 could form the micellar complexation with KP. KP could be more affinity to dissolve in the formulation more than penetrated into skin. Similarly, Arellano, *et al.*, reported that TW80 could decrease diclofenac permeation rate of diclofenac gel prepared with Carbopol gels containing 40%w/w of PG (24). They explained that due to a decrease in thermodynamic activity as a result of micellar complexation could change the barrier properties of the skin and the vehicle-stratum corneum partition coefficient (24).

The permeation flux and Q_{180min} of formulation containing 1%w/w L-LA were significantly higher than formulation without additive. The permeation flux of this formulation was 0.099 μg/cm^2 /min while the permeation flux of non-additive formulation was 0.059 μg/cm^2 /min. The Q_{180min} of 1%w/w L-LA formulation was 14.92 μg/cm^2 while the Q_{180min} of non-additive formulation was 8.85 μg/cm^2. These results are similar to the increased ketotifen flux when L-LA was used reported by Hiroyoki Nakamura, *et al.* (25). They explained that the enhancement effect of L-LA could be due to its hydroxyl group that may show a high affinity with ethanol, which played an important role in the enhancing effect, or may interact with drug in vehicle or in the skin barrier. In the another paper, Yoichi Kobayashi, *et al.*, estimated the action site of L-LA-ETOH-isopropyl myristate mixed system. They concluded that the site of action was on the aqueous domain of the stratum corneum and the lower layer of the skin (19). However, the detailed mechanism is not yet clear. Further studies are necessary to understand the mechanism and mode of action of skin-penetration-enhancing effect of this acid. Among these additives tested, L-LA had the highest enhancement effect for the permeation of KP through rat skin.

Table 14 Effects of mono-additives on KP Permeation flux and Q_{180min}

Time(min)	KP Permeated ($\mu g/cm^2$)			
	F-14 (Non-additive)	F-15 (PG)	F-16 (TW80)	F-17 (L- LA)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
10	0 ± 0	0 ± 0	0 ± 0	0 ± 0
20	0 ± 0	0 ± 0	0 ± 0	0 ± 0
30	0 ± 0	0 ± 0	0 ± 0	0 ± 0
60	2.810 ± 0.64	2.022 ± 0.50	0 ± 0	2.682 ± 0.47
90	4.037 ± 1.04	3.359 ± 0.91	0 ± 0	4.900 ± 2.05
120	7.156 ± 0.85	3.787 ± 0.98	2.340 ± 0.08	7.917 ± 2.30
180	8.854 ± 0.81	4.887 ± 1.41	2.935 ± 0.18	14.919 ± 4.46
Flux ($\mu g/cm^2/min$)	0.059 ± 0.01	0.031 ± 0.01	0.029 ± 0.00	0.099 ± 0.03
$Q_{180\ min}$ ($\mu g/cm^2$)	8.85 ± 0.81	4.89 ± 1.41	2.94 ± 0.18	14.92 ± 4.46

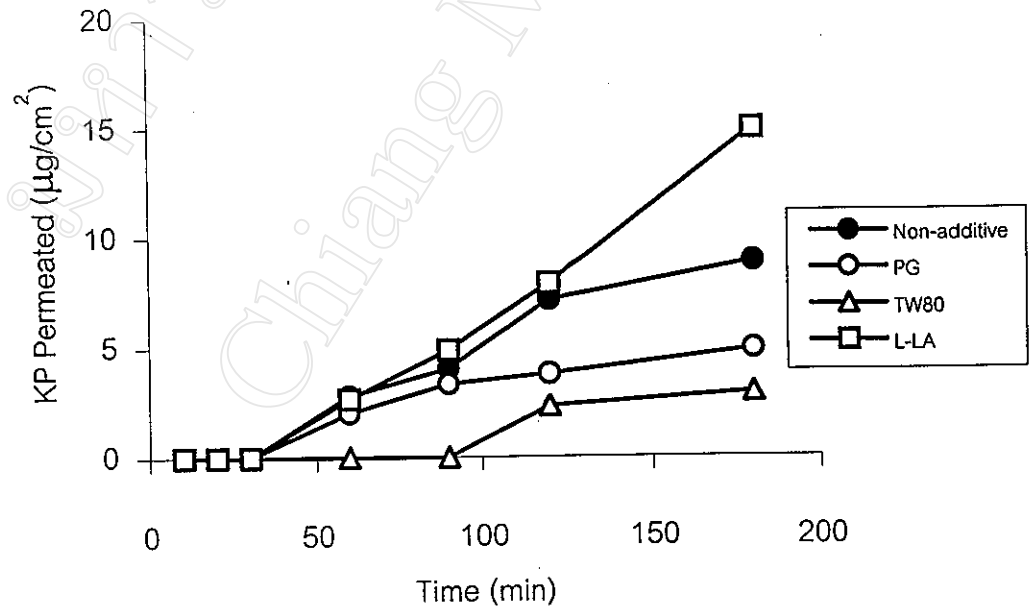

Figure 11 Effects of mono-additives on KP permeation through rat skin

3.5.2 Effects of combined additives on KP permeation flux and Q_{180min}

Table 15 and Figure 12 show the cumulative amount of KP permeated at each sampling time and the permeation parameters of all formulations containing combined additives. The KP permeation flux and Q_{180min} of formulation of binary additive (PG–L-LA) were similar to ternary additive (PG-TW80-L-LA) and were significantly higher than non-additive and another binary additive (PG-TW80).

The KP permeation flux and Q_{180min} of formulation added binary additive; PG-L-LA was the highest, similar to the ternary additive; PG-TW80-L-LA formulation and higher than the non-additive formulation but not significantly different from the formulation with only L-LA as additive. The domain enhancing effect of these combined additives could be due to L-LA. Some investigators suggested that hydroxyl group of L-LA may show a high affinity with ethanol that played an important role in the enhancing effect. And also L-LA may interact with drug in vehicle or in the skin barrier (25) and the site of action was may be on the aqueous domain of the stratum corneum and the lower layer of the skin (19). The KP permeation of ternary additive decrease may be due to the effect of TW80 to form micellar complexation with drug which could decrease the permeation of KP through the skin.

The KP permeation flux and Q_{180min} of formulation containing binary additive; PG-TW80 was the lowest, that similar to the non-additive formulation but it was higher than the formulation with only PG or TW80. This could be because TW80 disturbed the skin barrier property and promoted the PG enhancing effect.

For all combined additive formulations, the lag time of KP permeation was shorter than the non-additive formulation as shown in Figure 12. The combined additive could decrease the permeation lag time. Among the combined additives, PG-L-LA was a effective additive to increase KP permeation through rat skin.

Table 15 Effects of combined additives on KP Permeation flux and Q_{180min}

Time(min)	KP Permeated (μg/cm²)			
	F-14(Non-Additive)	F-18(PG-TW80)	F-19(PG-L-LA)	F-20(PG-TW80-L-LA)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
10	0 ± 0	1.532 ± 1.33	1.341 ± 2.32	0 ± 0
20	0 ± 0	1.813 ± 1.57	2.982 ± 2.65	3.031 ± 0.49
30	0 ± 0	2.076 ± 1.81	3.378 ± 3.01	3.794 ± 0.27
60	2.810 ± 0.64	3.425 ± 0.61	5.720 ± 2.41	4.591 ± 0.27
90	4.037 ± 1.04	4.174 ± 0.87	7.344 ± 3.75	5.896 ± 0.95
120	7.156 ± 0.85	5.264 ± 0.87	9.632 ± 4.91	8.773 ± 1.63
180	8.854 ± 0.81	8.178 ± 1.15	15.189 ± 6.34	13.102 ± 2.77
Flux (μg/cm²/min)	0.059 ± 0.01	0.038 ± 0.00	0.077 ± 0.03	0.068 ± 0.02
$Q_{180\ min}$ (μg/cm²)	8.854 ± 0.81	8.178 ± 1.15	15.189 ± 6.34	13.102 ± 2.77

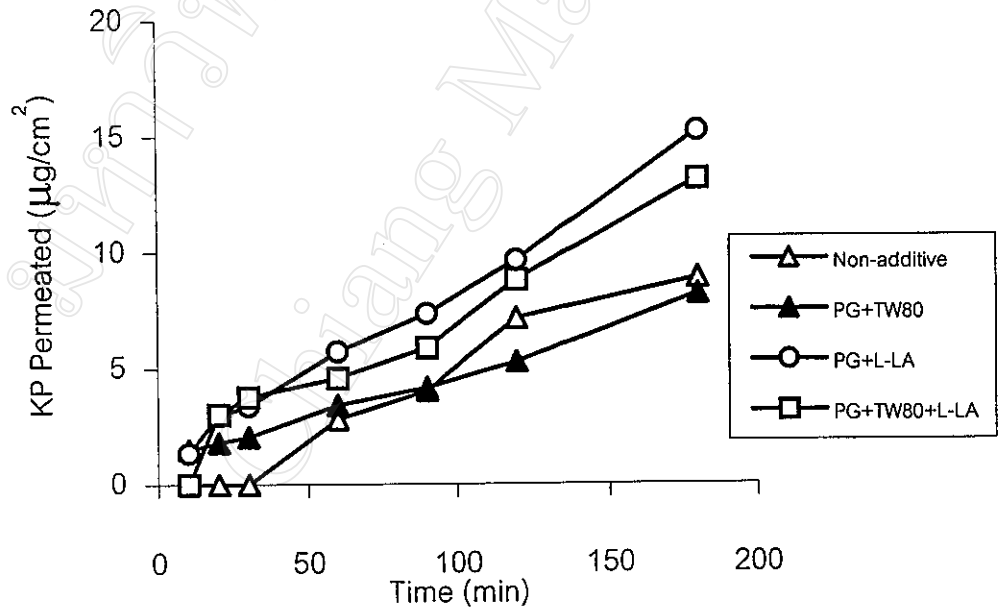

Figure 12 Effects of combined additives on KP permeation through rat skin

3.6 Comparison skin permeation of prepared and commercial KP gels

The prepared gel formulation which gave highest permeation flux, F-19, was further studied and compared with two commercial gels (Com1, Com2) containing the same concentration of KP and similar texture gel base. The mean of cumulative amount of KP permeated at each sampling time and the permeation parameters are shown in Figure 13 and Table 16. The permeation flux of KP for the two commercial products studied were significantly different from each another. The permeation flux was higher and faster for Com1 when compared with Com2. The permeation of KP from F-19 was higher than Com2 but slightly lower than Com1 (Figure 13). The difference of KP permeation may be due to the different ingredients used in the formulation of the products studied such as vehicle phase of each gel as well as additive added, even the KP concentration was the same in all gels used in this studies.

Table 16 Comparison skin permeation of prepared and commercial KP gels

Time (min)	KP Permeated ($\mu g/cm^2$)		
	Com1	Com2	F-19 KP gel
	Mean ± SD	Mean ± SD	Mean ± SD
10	4.951 ± 0.87	0 ± 0	1.341 ± 2.32
20	5.827 ± 0.81	0 ± 0	2.982 ± 2.65
30	6.798 ± 0.95	0.693 ± 1.20	3.378 ± 3.01
60	8.218 ± 0.55	0.877 ± 1.52	5.720 ± 2.41
90	10.089 ± 1.02	2.756 ± 0.72	7.344 ± 3.75
120	11.544 ± 1.57	3.900 ± 0.25	9.632 ± 4.91
180	16.906 ± 1.95	5.603 ± 1.41	15.189 ± 6.34
Flux ($\mu g/cm^2/min$)	0.066 ± 0.01	0.038 ± 0.00	0.077 ± 0.03
$Q_{180\ min}$ ($\mu g/cm^2$)	16.91 ± 1.95	5.6 ± 1.41	15.19 ± 6.34

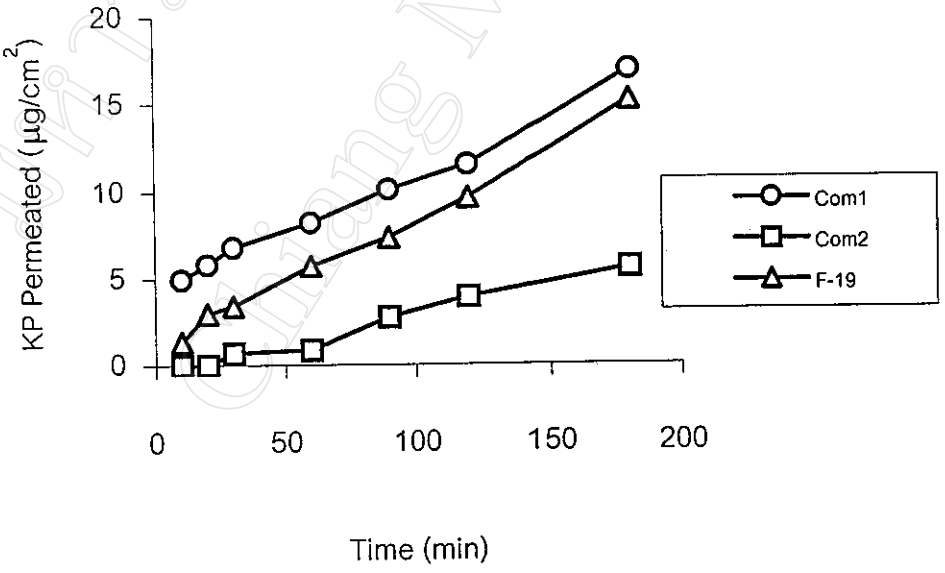

Figure 13 Comparison of prepared KP gel and commercial products on KP permeation through rat skin

3.7 Effects of temperature on KP released through cellophane membrane

In this study all the previous permeation studies were carried out at controlled room temperature of 25-26 °C. In order to estimate the permeation of KP gel at body temperature, the permeation of KP through a cellophane membrane at 25°C and 37°C was examined using the commercial KP gel 1. The mean of cumulative amount of KP released of each temperature and sampling time and other parameters are shown in Figure 14 and Table 17. The cumulative amount of KP released as a function of time at each temperature showed a strong linear correlation (R^2 >0.99), indicating the zero order kinetic drug released from gel. As the temperature increased from 25°C to 37°C the flux of KP increased from 6.14 to 7.76 μg/cm^2. This may due to increased solubility of KP at higher temperature. From the results of this study, the higher temperature, the higher KP permeation from all gels under study can be expected.

Table 17 Effects of temperature on KP released parameters

Time (min)	KP Released ($\mu g/cm^2$)	
	25 °C	37 °C
	Mean ± SD	Mean ± SD
10	43.560 ± 5.63	68.434 ± 3.19
20	91.590 ± 11.00	139.282 ± 16.60
30	148.746 ± 12.25	217.598 ± 19.97
60	320.897 ± 14.97	480.983 ± 8.49
90	490.979 ± 30.62	685.775 ± 44.76
120	683.219 ± 36.28	928.859 ± 56.88
180	1092.963 ± 48.72	1382.640 ± 72.93
Flux ($\mu g/cm^2/min$)	6.14 ± 0.26	7.761 ± 0.39
Correlation	0.9976 ± 0.00	0.9986 ± 0.00
$Q_{180\ min}$ ($\mu g/cm^2$)	1092.96 ± 48.72	1382.64 ± 72.93

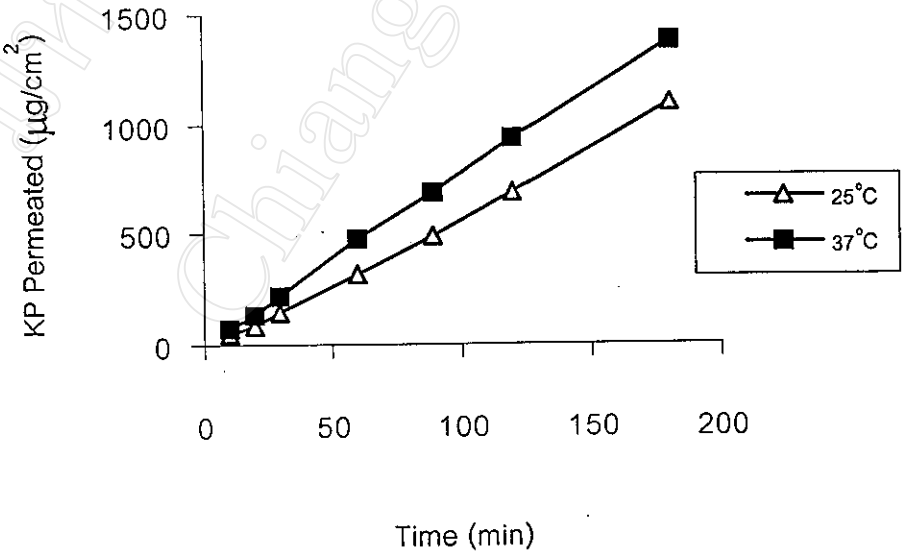

Figure 14 Effects of temperature on KP released through cellophane membrane

4 Stability tests

4.1 Effects of aging on KP content and physical properties of gels

Stability of the product is one of the great concern in the formulation of KP gel. In this investigation the gels were kept under long-term test at room temperature for six months and kept under stress conditions of heating and cooling for six cycles. The drug content, viscosity, and pH of gels were used to evaluate their effect on the gels. The mean drug content, pH, and viscosity for each formulation are displayed in Table 18, Table 19, and Table 20. The stability studies showed that KP content of all KP gels under stress conditions changed in range from –9.89 to 7.23 % of initial KP content. The KP content in most gels tested under this condition were less than initial. It may be due to KP gel being kept at 2-8 °C 48 hr then switched to 45°C 48 hr for six cycles; the KP content could decrease because the KP was not stable at higher temperatures. At the same time, in some gels, the KP content increased. This may be due to the evaporation of ETOH. Similar results were found for gels kept at room temperature for six months, but the loss of KP content in gel was less than the under stress test. The KP contents of Com1 under stress condition and at room temperature for six months were not different. The percentage of KP change from initial was a decrease of 4.51 % and 4.16% for each condition used. Similarly, the KP contents of Com2 were increased 1.22% and 1.70%. For prepared KP gels, more variation of KP contents from two conditions were observed. For example, KP content of F-14 under stress conditions was lost 7.00%, while at room temperature six months test the KP content increased 3.68%. The storage temperature and the storage conditions of KP gels might affect KP content in gels. It was found that the pH of KP gels under stress condition changed in a range of -5.77% to 6.90% of initial pH and the viscosity changed in a range of -7.22% to 9.52 % of initial viscosity. However, of all indicators; drug content, pH, and viscosity, for evaluating KP gel stability were found to have not changed than ±10% of initial, these were acceptable. The best stable formulation was F-18 (PG-TW80), it was found % changed of KP content under stress condition, % changed KP content after kept at room temperature for six months,

% changed of pH of the formulation, and % changed of viscosity were 1.64, 1.32, 0.81, and 1.29 % respectively. However, the stability of all KP gels were observed.

Table 18 Effects of storage conditions on KP content (%w/w)

No.	Factor studies	KP Content (%w/w)				
		Initial Mean±SD	6 cycles Mean±SD	%changed after 6 cycles	6 months Mean±SD	%changed after 6 months
F-1	CBP2020 3%	2.50±0.01	2.26±0.05	-9.60	2.49±0.07	-0.40
F-2	CBP2020 2%	2.34±0.03	2.25±0.01	-3.85	2.40±0.05	+2.56
F-3	CBP2020 1.5%	2.43±0.09	2.22±0.02	-8.64	2.40±0.04	-1.23
F-4	CBP980 3%	2.55±0.18	2.33±0.04	-8.63	2.47±0.06	-3.14
F-5	CBP980 2%	2.65±0.06	2.53±0.06	-4.53	2.45±0.06	-7.55
F-6	CBP980 1.5%	2.49±0.14	2.49±0.07	+0.00	2.25±0.02	-9.64
F-7	HPMC 3%	2.45±0.08	2.46±0.04	+0.41	2.50±0.07	+2.01
F-8	HPMC 2% ETOH 35.5%	2.35±0.08	2.52±0.13	+7.23	2.44±0.01	+3.81
F-9	HPMC 2% ETOH 30%	2.43±0.21	2.27±0.08	-6.58	2.41±0.05	-0.69
F-10	HPMC 2% ETOH 40%	2.41±0.05	2.51±0.06	+4.15	2.50±0.08	+3.67
F-11	HPMC 2.5% pH 3.4	2.63±0.11	2.37±0.02	-9.89	2.47±0.03	-6.26
F-12	HPMC 2.5% pH 5.7	2.41±0.24	2.31±0.04	-4.15	2.50±0.05	+3.70
F-13	HPMC 2.5% pH 7.0	2.48±0.06	2.25±0.02	-9.27	2.31±0.03	-6.72
F-14	HPMC 3% Non-additive	2.43±0.07	2.26±0.03	-7.00	2.52±0.02	+3.68
F-15	HPMC 3% PG	2.41±0.06	2.48±0.13	+2.90	2.51±0.02	+4.34
F-16	HPMC 3% TW80	2.53±0.15	2.43±0.16	-3.95	2.61±0.06	+3.17
F-17	HPMC 3% L-LA	2.54±0.12	2.40±0.10	-5.51	2.51±0.03	-1.16
F-18	HPMC 3% PG-TW80	2.44±0.04	2.48±0.09	+1.64	2.47±0.04	+1.32
F-19	HPMC 3% PG-L-LA	2.56±0.06	2.38±0.12	-7.03	2.32±0.08	-9.25
F-20	HPMC 3% PG-TW80-L-LA	2.48±0.05	2.27±0.13	-8.47	2.49±0.07	+0.47
Commercial KP gel1		2.66±0.05	2.54±0.06	-4.51	2.55±0.06	-4.16
Commercial KP gel2		2.45±0.10	2.48±0.02	+1.22	2.49±0.08	+1.70

Table 19 Effects of storage under stress conditions on pH of gels

No.	Factor studies	pH		
		initial Mean±SD	6 cycles Mean±SD	%changed after 6 cycles
F-1	CBP2020 3%	5.41±0.01	5.40±0.02	-0.18
F-2	CBP2020 2%	5.36±0.03	5.35±0.03	-0.25
F-3	CBP2020 1.5%	5.79±0.01	5.46±0.07	-5.70
F-4	CBP980 3%	6.48±0.01	6.53±0.01	+0.67
F-5	CBP980 2%	6.15±0.01	6.11±0.08	-0.60
F-6	CBP980 1.5%	6.30±0.01	6.30±0.01	+0.05
F-7	HPMC 3%	7.13±0.01	7.29±0.02	+2.25
F-8	HPMC 2% ETOH 35.5%	5.62±0.08	5.52±0.02	-1.84
F-9	HPMC 2% ETOH 30%	6.38±0.02	6.46±0.01	+1.20
F-10	HPMC 2% ETOH 40%	6.09±0.03	6.51±0.01	+6.90
F-11	HPMC 2.5% pH 3.4	3.36±0.03	3.29±0.01	-2.18
F-12	HPMC 2.5% pH 5.7	5.67±0.02	5.74±0.02	+1.23
F-13	HPMC 2.5% pH 7.0	6.97±0.03	7.21±0.02	+3.40
F-14	HPMC 3% Non-additive	5.65±0.06	5.60±0.01	-0.88
F-15	HPMC 3% PG	7.34±0.02	7.45±0.03	+1.50
F-16	HPMC 3% TW80	6.74±0.01	6.93±0.01	+2.87
F-17	HPMC 3% L-LA	5.38±0.03	5.07±0.01	-5.77
F-18	HPMC 3% PG-TW80	5.75±0.02	5.71±0.01	-0.81
F-19	HPMC 3% PG-L-LA	5.49±0.07	5.31±0.03	-3.22
F-20	HPMC 3% PG-TW80-L-LA	5.23±0.02	5.17±0.02	-1.15
Commercial KP gel1		5.76±0.02	5.55±0.05	-3.70
Commercial KP gel2		6.55±0.03	6.61±0.06	+0.97

Table 20 Effects of storage under stress conditions on viscosity of gels

No.	Factor studies	Viscosity (cP)		
		initial Mean±SD	6 cycles Mean±SD	%changed after 6 cycles
F-1	CBP2020 3%	16980±500	16527±172	-2.67
F-2	CBP2020 2%	12413±390	12640±164	+1.83
F-3	CBP2020 1.5%	9720±35	9390±171	-3.40
F-4	CBP980 3%	19120±695	18887±1040	-1.22
F-5	CBP980 2%	15953±12	15753±105	-1.25
F-6	CBP980 1.5%	10027±284	10487±462	+4.59
F-7	HPMC 3%	12460±365	11560±310	-7.22
F-8	HPMC 2% ETOH 35.5%	3647±50	3547±50	-2.74
F-9	HPMC 2% ETOH 30%	3707±155	3913±31	+5.58
F-10	HPMC 2% ETOH 40%	3747±12	3910±26	+4.36
F-11	HPMC 2.5% pH 3.4	6507±70	6493±155	-0.20
F-12	HPMC 2.5% pH 5.7	6573±12	6600±203	+0.41
F-13	HPMC 2.5% pH 7.0	6273±81	6633±397	+5.74
F-14	HPMC 3% Non-additive	13173±153	13687±277	+3.90
F-15	HPMC 3% PG	14720±139	15093±114	+2.54
F-16	HPMC 3% TW80	15360±164	15787±101	+2.78
F-17	HPMC 3% L-LA	15447±42	16480±269	+6.69
F-18	HPMC 3% PG-TW80	16040±72	15833±204	-1.29
F-19	HPMC 3% PG-L-LA	14707±95	16107±81	+9.52
F-20	HPMC 3% PG-TW80-L-LA	12960±20	12120±40	-6.48
Commercial KP gel1		5867±50	5850±30	-0.28
Commercial KP gel2		10787±99	10820±122	+0.31

4.2 Effects of aging on permeation of KP.

KP gel F-19 and Com1 were selected for stability studies under the stress conditions of heating cooling for six cycles (H&C). The KP permeation parameters of both gels are shown in Table 21 and Table 22. The premeation profiles are shown in Figure 15 and Figure 16. There were slightly changed. KP permeation flux and Q_{180min} of F-19 after stress conditions were lower than initial, but KP permeation flux of Com 1 was the same as initial and Q_{180min} was slightly decreased.

The KP permeation of KP gel before and after stress conditions was investigated to confirm the stability of gels on KP permeation. The KP permeation flux and Q_{180min} of F-19 decreased but not significantly. The decrease of KP permeation from F-19 after stress conditions may be due to its KP content loss of 7.03% from initial that could decrease the KP permeation. In similar results, Q_{180min} of Com1 was slightly decreased because its KP content decreased by 4.51%, but the same flux was observed. In conclusion, KP gels after passing through stress conditions showed no pronounced effect on KP permeation. However, the storage condition of KP gel must be considerated.

Table 21 Effects of aging under stress conditions of Com1 on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)	
	RT	H&C
	Mean ± SD	Mean ± SD
10	4.951 ± 0.87	3.992 ± 0.37
20	5.827 ± 0.81	5.088 ± 0.94
30	6.798 ± 0.95	6.927 ± 2.71
60	8.218 ± 0.55	7.892 ± 3.00
90	10.089 ± 1.02	9.688 ± 3.32
120	11.544 ± 1.57	11.713 ± 2.66
180	16.906 ± 1.95	15.777 ± 0.09
Flux ($\mu g/cm^2/min$)	0.066 ± 0.01	0.066 ± 0.01
$Q_{180\ min}$ ($\mu g/cm^2$)	16.91 ± 1.95	15.78 ± 0.09

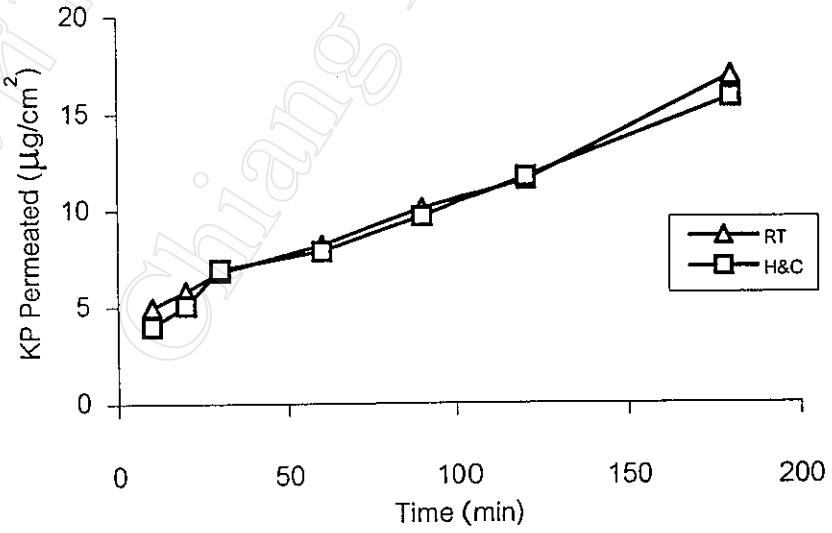

Figure 15 Effects of aging under stress conditions(H&C)of Com1 on the KP permeation profile

Table 22 Effects of aging under stress conditions(H&C) of F-19 on KP permeation flux and Q_{180min}

Time (min)	KP Permeated ($\mu g/cm^2$)	
	RT	H&C
	Mean ± SD	Mean ± SD
10	1.341 ± 2.32	0 ± 0
20	2.982 ± 2.65	0 ± 0
30	3.378 ± 3.01	0 ± 0
60	5.720 ± 2.41	3.329 ± 0.77
90	7.344 ± 3.75	4.202 ± 0.32
120	9.632 ± 4.91	6.180 ± 1.02
180	15.189 ± 6.34	10.291 ± 0.80
Flux ($\mu g/cm^2/min$)	0.077 ± 0.03	0.065 ± 0.00
$Q_{180\ min}$ ($\mu g/cm^2$)	15.189 ± 6.34	10.291 ± 0.80

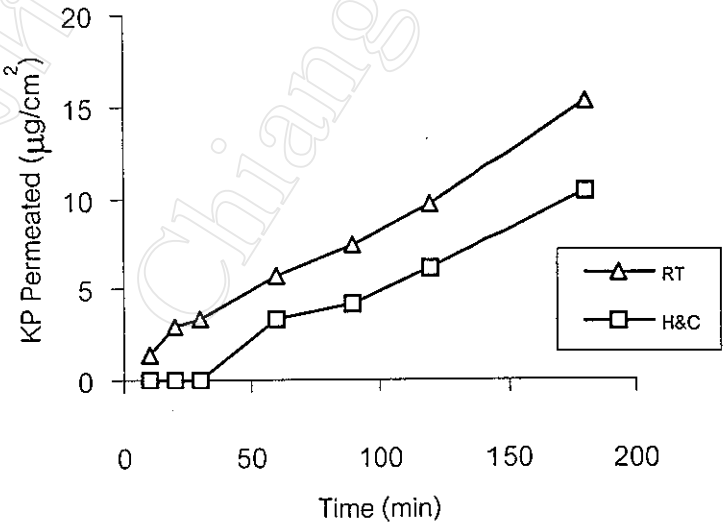

Figure 16 Effects of aging under stress conditions(H&C)of F-19 on the KP permeation profile