CHAPTER IV

Discussion and Conclusion

Evaluation of Drug Powder

Diclofenac is used in commercial topical products in two salt forms , diclofenac sodium and diethylammonium salts. The drug is not yet specified in current The United State Pharmacopoeia (USP XXII,1989). However DS is specified in Martindale The Extra Pharmacopoeia (Gennaro ; A.R.,1990). Chemical characteristics of DS can also be found in some journals and textbooks (Adeyoyeand Li,1990 , Budavari; S. ,1989). On the contrary, DE almost have no published information. In order to differentiate these two salts forms, both IR spectra of drugs and physical appearances such as crystal forms and shape under scanning electron microscope were investigated.

Microscopic appearances of DS and DE were different in both size and shape of crystal particle. These differences maybe due to the difference in molecular arrangement of crystal, and other factors such as production process of these two salts.

The IR spectra of DS and DE were different in some band regions. Basic bands of two salts of diclofenac

were band A in 3350-3310 cm^{-1} region (N-H stretching vibration), bandB in 3100-3000 cm^{-1} region (aromatic C-H stretching vibration), band E in 1600-1550 cm^{-1} region (asymmetrical carboxyl stretching vibration) and band F in 1400 cm^{-1} (symetrical carboxyl stretching vibration). Additional bands of DE were noted. Specific band of DE were band C in region of 3000-2840 cm^{-1} (C-H stretching vibration of alkane) ,band D in region of 2600-2200 cm^{-1} (N-H stretching vibration of amine salts) and broad spectrum which rised from the base line of secondary amine salt were in 3000-2273 cm^{-1} region. Band C and D in DE IR spectra indicates ethyl groups and amine salts of the diethylamine part of the molecule.

Solubility Determination

Since diclofenac salts are poorly soluble in water (Nishihata et al, 1988), solubility data of diclofenac is not only necessary to characterize the salt forms of drug but also essential for preformulation. DS dose in topical preparation is normally 1 g. per 100 g. base. DE dose in topical preparation is 1.16 g. per 100 g. base which is equivalent to DS 1 g. per 100 g. base. At least, a 1 g. and 1.16 g. of DS and DE, respectively, should be completely dissolved in desired phase of preparation in order to ensure that drug does not precipitate in finished preparation. In this study, DS and DE were firstly dissolved in water phase of cream, oil-

water gel which they could later partition to oil phase, and hydrophilic gel.

The solubility of DS and DE in water increased as the pH of water increased but at the same pH, DS was more soluble than DE. DS and DE are salts of organic carboxylic compounds which ionize to two ionic species, cationic specie and anionic specie, when they dissolve in water (Tomida et al., 1987) These species are surrounded by water molecule. The more ionized of diclofenac the more increasing of its solubility. At high concentration of hydroxide ion, diclofenac is more ionized than at low pH so that the solubility of diclofenac was increased by increasing the pH of water.

Sodium ion is smaller than diethylammonium ion. And electric charge per ion of sodium ion is more than that of diethylammonium ion, sodium ion is more easily solvated by water than diethylammonium ion. Therefore, sodium ion can be ionized from DS more than diethylammonium ion ionized from DE, which makes DS more soluble in water than DE.

In this study, the general rank order of solubility of both DS and DE in solvent was : sorbitol solution < isopropyl alcohol < glycerin < ethanol < propylene glycol < methanol. Sorbitol solution contains approximately 70 % of sorbitol, an hexose sugar, and 30 % of water. Most of

water molecules are already attached to hydroxy groups around sorbitol molecule , it is causing few free molecule water to dissolved or ionized diclofenac. For other hydroxy compound, such as isopropyl alcohol, glycerin, propylene glycol and methanol which have two parts in the molecule, hydrophobic part (hydrocarbon skeleton) and polar part (hydroxy group). In these systems, ion of diclofenac molecule was surrounded by hydroxy group of solvent. The solubility of diclofenac was increased as the increasing of polar group per carbon atom ratio : isopropyl alcohol (1:3) < ethanol (1:2) < propylene glycol (1:1.5) < methanol (1:1), except for glycerin (1:1). The latter result may be affected by the viscosity of glycerin as defined by Stokes-Einstein equation

$$D = kT/6\eta r$$

where D is diffusion coefficient of drug molecule, k is Boltzmann contant, T is absolute temperature, η is vehicle viscosity and r is molecular radius. An increase in vehicle's viscosity will decrease the drug's diffusion coefficient as well as dissolution. The viscosity of glycerin is 1460 cps at 20 ^{o}C and 954 cps at 25 ^{o}C, whereas the viscosity of methanol is 0.60 and 0.54 cps at 20 and 25 ^{o}C, respectively. (Godfrey, 1972., Skoog, 1985)

One gram of DS may be almost completely dissolve in 100 g. of pH 7 water in topical praparation at 35 ^{o}C but it

may precipitate at room temperature. Addition of solvent is needed in DS topical preparation in order to prevent drug from precipitation. Certainly 1.16 grams of DE can not be completely dissolved in 100 g. of pH 7 buffer 35 ^{o}C. Co-solvent should be definitely added in DE topical preparation. Though the highest of drug solubility, methanol still cannot be used as a co-solvent because it is toxic for human used (Reynold, 1989). Besides methanol is also used as an eluent in high-performance liquid chromatographical analysis of this drug. The selected solvents as co-solvent in formulation were propylene glycol and isopropyl alcohol. Not only propylene glycol was used in formulation to increase drug solubility and prevent drug from precipitation but it alsoacted as humectant in formulation. Isopropyl alcohol was normally used in hydrophilic gel, emulsion and oil-water gel as co-solvent and have cooling effect.

Evaluation of Diclofenac Stability

Stability data of diclofenac in water pH 5-9 were determined in order to choose the optimun pH to stabilize the drug. In stability studies, Sorensen phosphate buffer system (Flynn, 1980) was chosen because two stock solutions of this system can be mixed at different ratio to form pH 5, 6, 7, 8 and 9 buffers by using a pH meter. The same buffer species were used in order to decrease the effect of buffer species on

degradation mechanism of diclofenac (Connors, 1981; Flynn, 1980).

1. Effect of pH on Diclofenac Stability

Degradation rates of diclofenac were calculated from the slope of a plot between the remained concentration of diclofenac versus time of storage. At the same temperature, diclofenac in several pH buffers degraded with an unequal rate. Degradation rate of DE and DS in pH 5,6,7,8 and 9 buffers at 35, 45, 55 and 65 ^{o}C after 5 months of storage were illustrated in Table 23. A plot of degradation rate versus buffer pH of both salts were shown in Figures 43 and 44.

Degradation rate of diclofenac in pH 5 was higher than the others at the same temperature condition whereas diclofenac in pH 6, 7, 8 and 9 were closely equal. The general rank order of the stability of DS was : pH 5 < pH 6 ~ pH 7 < pH 8 < pH 9. The general rank order of the stability of DE was : pH 5 < pH 6 ~ pH 7 < pH 8 ~ pH 9.

Degradation reaction of diclofenac in solution was related to the pH of solvent. The lower of the pH of buffer, the more increasing of degradation. Hydronium ion in solution may act at diclofenac molecule to accerate the degradation reaction of diclofenac. Chemical reaction of diclofenac degradation is suggested as in Figure 45.

Table 23 Degradation rate of DS and DE in pH 5,6,7,8 and 9 buffer solution at 35, 45, 55 and 65 C

Drug	Temp.	pH 5	pH 6	pH 7	pH 8	pH 9
DS	35	1.18730	0.10730	0.13530	0.16125	0.14814
	45	2.34254	0.33658	0.38234	0.31475	0.30072
	55	3.07574	1.20794	1.17805	1.00521	0.90946
	65	3.00334	1.27343	1.29031	1.10116	1.00254
DE	35	0.66632	0.15388	0.18506	0.10628	0.15993
	45	0.60504	0.27681	0.38259	0.24162	0.24384
	55	1.39940	1.07015	1.02630	0.93184	0.77759
	65	1.52482	1.24504	1.19530	0.98353	0.94144

Remark : Conc. = mole/months * 1.00E5

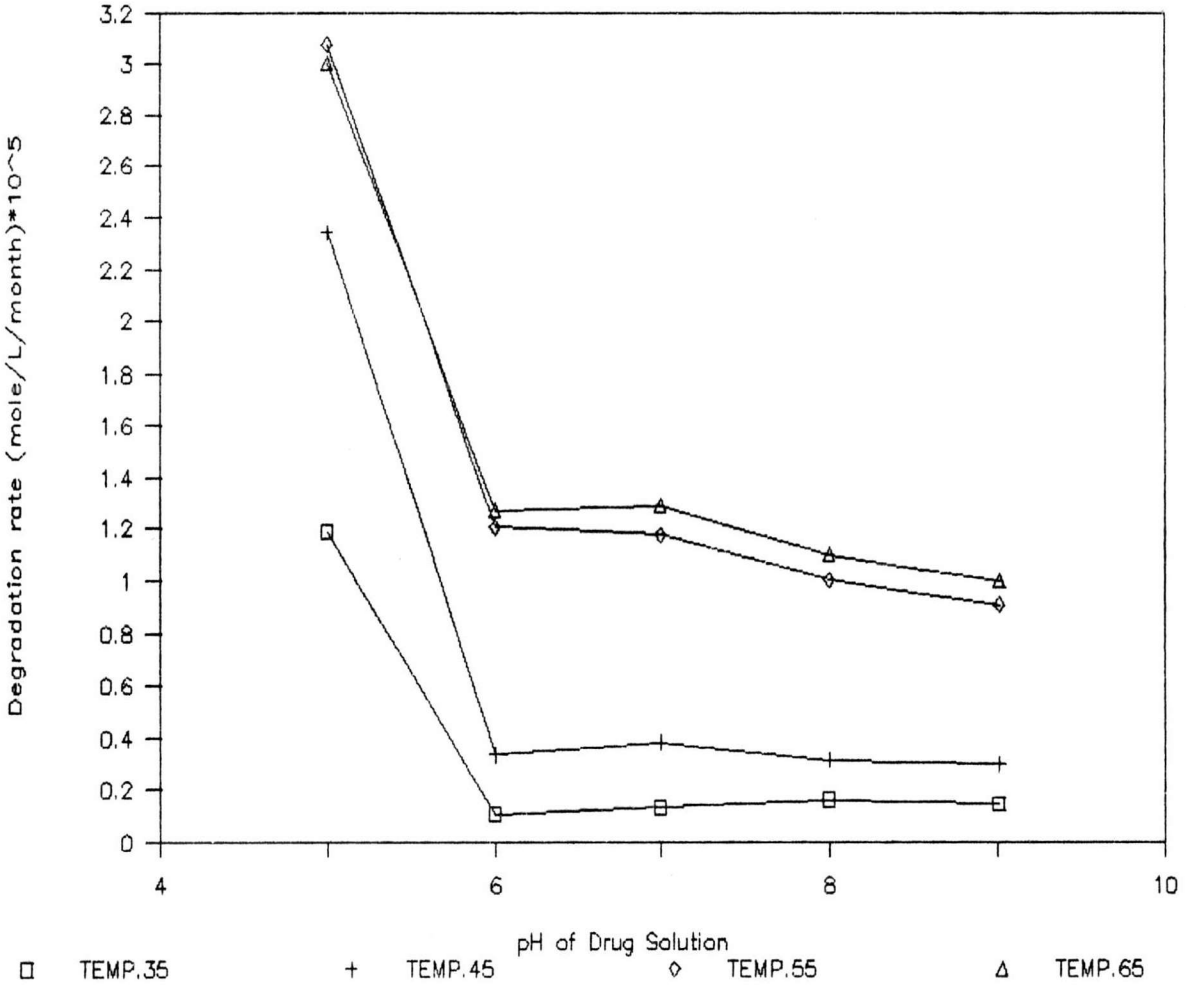


Figure 43 pH-rate profile for the degradation of DS at 35, 45, 55 and 65 ^{o}C

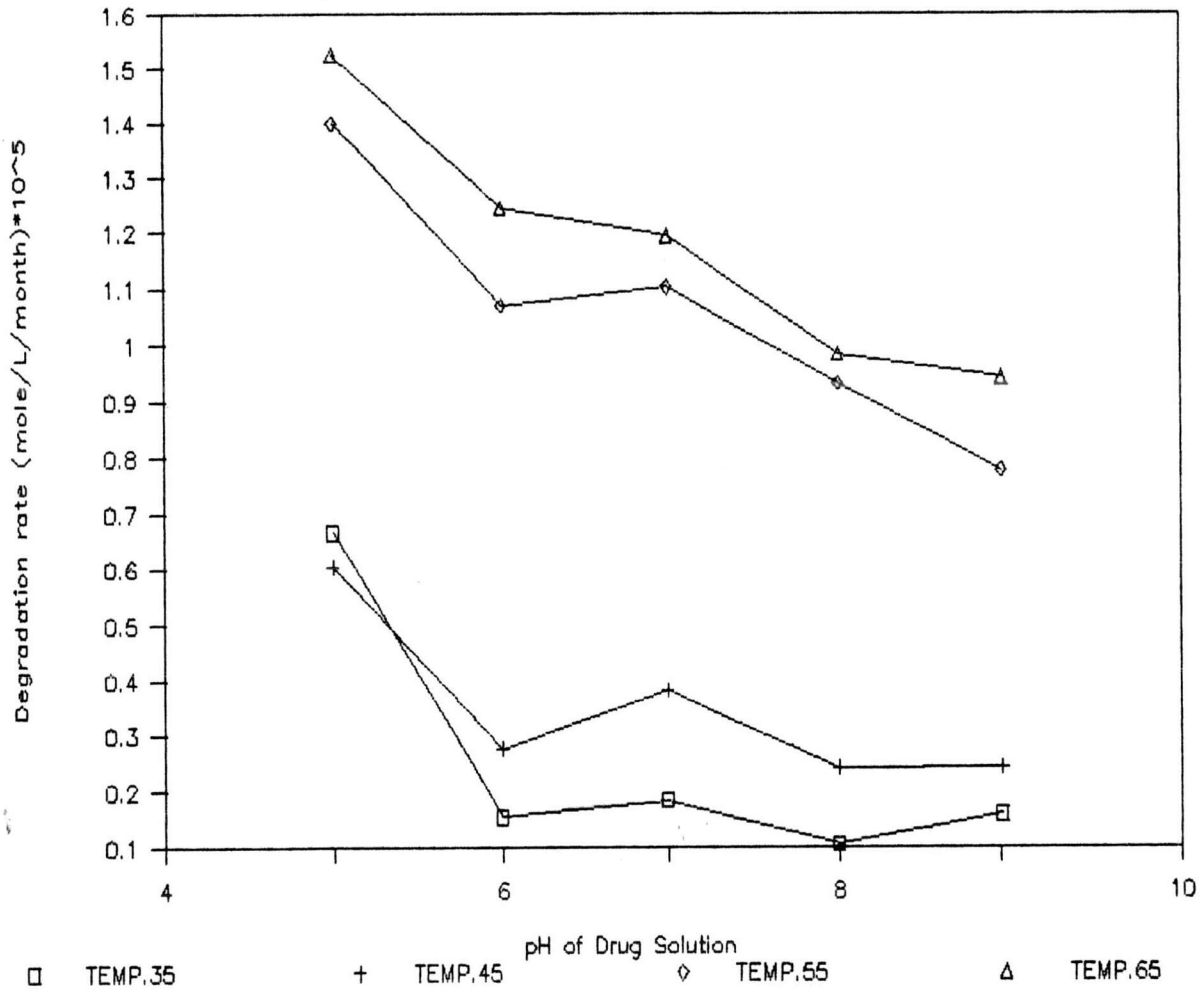


Figure 44 pH-rate profile for the degradation of DE at 35, 45, 55 and 65 ^{o}C

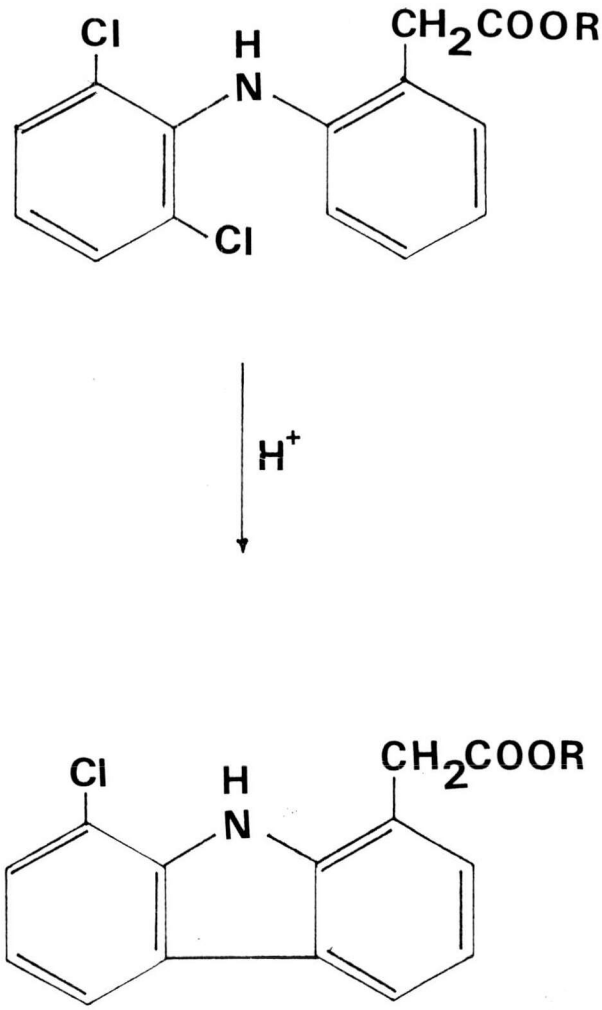


Figure 45 Schematic illustration of chemical degradation reaction mechanism of diclofanac

(modified from Connors, 1986)

2. Effect of Temperature on Diclofenac Stability

Diclofenac in each pH solution was tested for their stability at 35, 45, 55 and 65 ^{o}C. If the temperature of testing were fixed, Diclofenac degraded with an unequal rate depend on their pH of solution.

Degradation rate of diclofenac in each pH solution were related to temperature. The increasing of temperature leads to the increasing of degradation rate of diclofenac as shown in Arrhenius's plot. These results may explained by the Kinetic Molecular Theory (KMT) and Arrhenuis equation (Stella, 1986);

$$k = Ae^{-Ea/RT}$$

$$\log k = \log A - Ea/2.303RT$$

where k is the observed order rate constant for the reaction, A is the collision number of reaction, Ea is the observed energy of activation, R is the gas constant (1.987 cal/mole/degree), and T is an absolute degree (^{o} C+273 Kelvin). Chemical reaction occurs when molecules come together with sufficient energy to overcome the free energy of reaction. Most energy in a molecule comes from kinetic or translational energy, which along with other

forms of molecular energy, is temperature dependent. As the temperature increases, it causes diclofenac molecule to move with more velocity, more kinetic, and more frequency of collision, that causes more degradation reaction of diclofenac.

Logarithm of degradation rate was plotted versus the recipocal of absolute temperature of storage following Arrhenius equation, as shown in Figures 46 and 47 . The enegy of activation of diclofenac in several pH buffer could be derived from slope of the Arrhenius's plot and illustrated in Table 24. The Arrhenius plot's R-Squre of DS in pH 5, 6, 7, 8 and 9 solution were 0.811903, 0.919895, 0.928300, 0.929654 and 0.933403, respectively. The Arrhenius plot's R-Squre of DE in pH 5, 6, 7, 8 and 9 solution were 0.779078, 0.929079, 0.931155, 0.921133 and 0.932709, respectively. The Ea of DS and DE in pH 5 were lower than other pH's which have closely Ea value. These results confirmed that DS and DE in pH 5 solution were easierly degraded than in other pH's which remained more than 90 percent of drug content after 5 months of storage at 35 ^{o}C.

Preparation Appearance Evaluation

In order to prepare an optimum topical preparation of diclofenac , with enough drug solubility , good drug stability and gentle for skin used, pH 7 buffer was

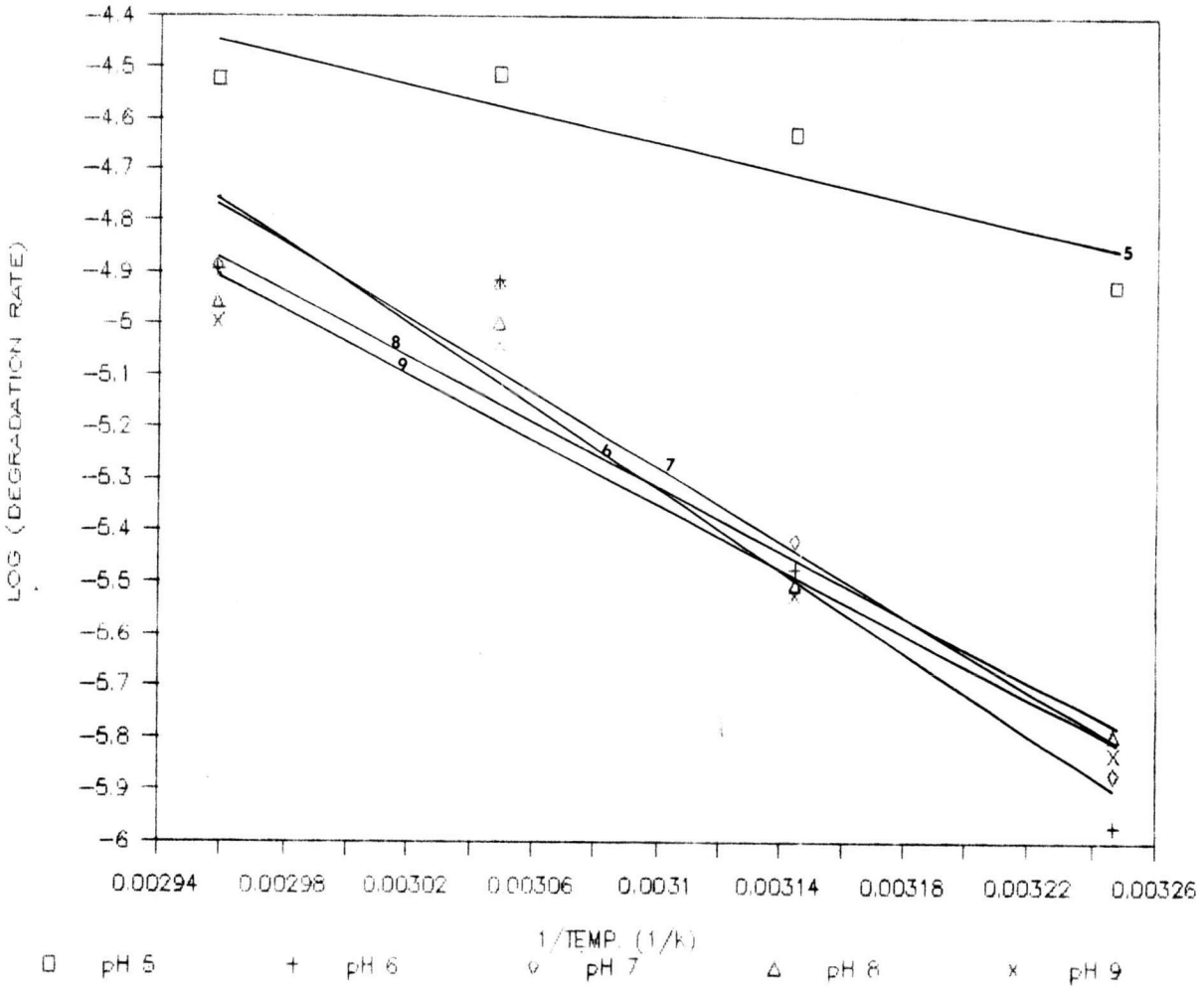


Figure 46 Arrhenius plots for the degradation of DS at pH 5, 6, 7, 8 and 9

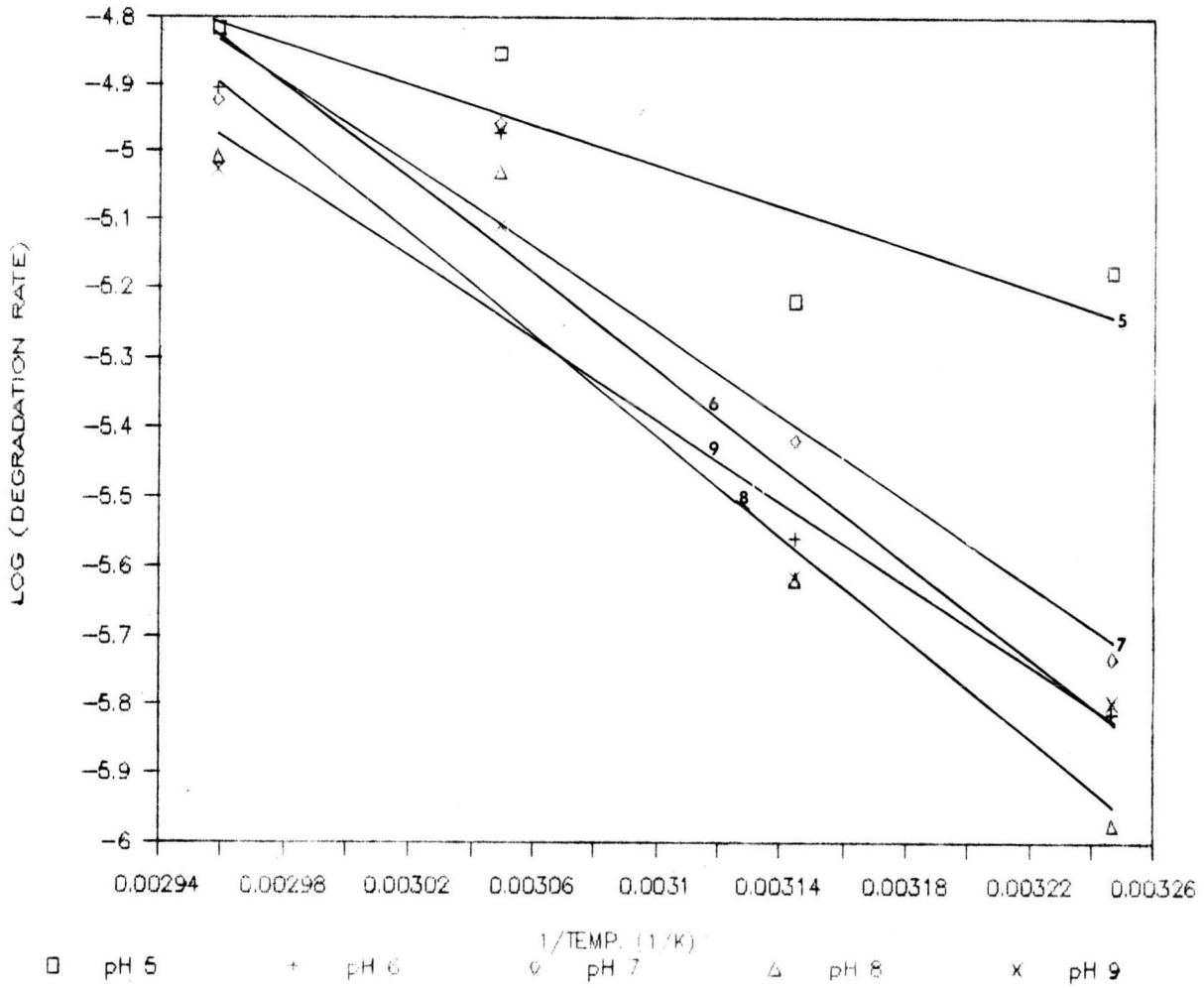


Figure 47 Arrhenius plots for the degradation of DE at pH 5, 6, 7, 8 and 9

Table 24 Activated energy (Ea) of diclofenac in pH 5, 6, 7, 8 and 9 buffer solution
(cal/mole)

pH	DS	DE
5	6410.15	6843.77
6	18125.91	15818.80
7	16435.93	13841.67
8	14390.43	16689.63
9	14224.87	13421.63

Remark : cal/mole

chosen as a solvent and/or aqueous phase of the preparation althrough pH of the skin is approximately 5.5. A pH 6 buffer may used as a solvent and/or aqueous phase of the preparation but if a pH of the praparation are decreased during storing, the drug will be rapidly degraded. IPA was chosen as a co-solvent and had cooling effects instead of ethanol because IPA possessed good smell and commonly used in commercial product. Placebo preparations were also prepared in this study if the appearance of placebo preparation and preparation which contained the same base components and drug was different.

1. Creams

Diclofenac creams which using cetomacrogol 1000 as emulsifier were smooth and fine homogenized texture and had no phase separation after freeze-thaw cycles whereas creams which using Span and Tween as emulsifier were coarse texture and segregrated after freeze-thaw cycles. These results may be affected by using different emusifier.

All cream preparations in this study were oil in water type because high HLB (hydrophilic-lipophilic balance) of emulsifiers and low ratio of oil phase compare with water phase. Emulsifiers that were used in all cream preparations are nonionic surfactants.

Nonionic surfactant added to the two-phase system of water and hydrocarbon preferentially adsorbs at the interface, forming an adsorbed monolayer (Shinoda,1986).

There are two reasons why different emulsifier leads the creams to have the different properties. One reason, the adsorbed monolayer of Tween and Span may be weaker than an adsorbed monolayer of cetomacrogol so that oil globule in Tween and Span cream can come toward other globule and coaslescent easier than oil globule in cetomacrogol cream. The other reason is the rigidity of oil globule. Cetomacrogol is a solid state emulsifier whereas Tween and Span are liquid. Oil globules which used cetomacrogol as emulsifier were harder than in Tween and Span cream. The increaseing rigidity of oil globule contributes more difficulty in coalescence.

2. Hydrophilic Gels

Placebo carbopol gel preparations contained 10-25 mL of propylene glycol were clear,transparent and viscous. When diclofenac was in the formulation of 25 mL of propylene glycol, similarly clear and transparent but more liquid was obtained. This result was affected by the incompatibility of drug and carbopol. Diclofenac may act at the carboxylic group of carbopol chain that decrease the hydration of carbopol and decrease the viscosity (Pillai et al, 1988). For another reason, the viscosity of

carbopol gel is also reduced in the presence of strong electrolyte (American Pharmaceutical Association , 1986). Diclofenac salts acted as electrolyte at pH 7 buffer therefore the viscosity of carbopol gel are reduced. Thus carbopol is not suitable as gelling agent for diclofenac.

Clear liquid gel with white precipitate was obtained when formulated with lesser amount of propylene glycol. The lower viscosity of gel could be explained as previously mentioned. White precipitates may be drug which precipitated because of not enough co-solvent in these systems. For completely solubilized diclofenac in carbopol gel, 25 mL of propylene glycol was needed.

Increasing the carbopol content in preparation did not increase the viscosity of carbopol gel which contained diclofenac. Neutralizing agent, either 10 % sodium hydroxide solution or triethanolamine, that uses for neutralizing carbopol had the same effect on preparation appearance.

A 14 percent of propylene glycol in sodium CMC and poloxamer gel was sufficient to absolutely dissolve diclofenac. After diclofenac was incorporated, poloxamer gels which contained 20 percent of poloxamer were liquid gel. Viscous diclofenac and poloxamer gel should contain more than 25 percent of poloxamer.

All ingredients in diclofenac emulsion preparations were in liquid state. Span 20 and Tween 20 were chosen as emulsifier in praparation. However they could not prevent emulsion from phase separation after freeze-thaw cycles. The weakness of adsorbs monolayer of Span and Tween may be reason. Emulsion which prepared in three different methods did not show different physical appearance. It could be concluded that pepparation method did not affect the appearance of emulsion.

Silicone oil and mineral oil could not be used in oil-water gel preparation because the finished preparation were not uniform and segregate after freeze-thaw cycle. These result indicated that PHC and poloxamer can not solubilized these oil. PHC is an nonionic emulsifier and solubilizer, it forms micelle in solution. Oil-water gel preparations which contained less than 5 percent of castor oil were transparent. This results may affectd by the completely solubilization of castor oil in micelle. When increasing the amount of castor oil to more than 5 percent, the gel were began turbid. This result may cause by too high amount of castor oil to soluble in the micelle, so that the excess oil phase can disperse in the external phase or aqueous phase, as a droplet with adsorbed monolayer of PHC. Oil droplets might be larger than micelle. They could scatter light as the Tyndall effects of colloids so that the preparation were turbid in appearance.

In-Vitro Study of Preparation

The therapeutic efficacy of topical administration drug depends on two steps : 1) drug released from topical base, 2) drug which released from base penetrate through the skin to target site or to blood circulation and then go to target site (Osborne et al., 1990). In research and development of topical preparation, in-vitro release study or the studying of drug release through the membrane is a suitable step to test for a good preparation. A preparation that has good drug release may expectly exhibit good skin penetration.

1. Creams

Student's t distribution test of hypothesis and significance of these creams were shown in Tables 25 and 26.

1.1 Effect of Percent of Oil Phase in o/w Cream on Drug Release.

A plot of percent drug released versus time was shown in Figures 48 and 49. DS in formulations 7 and DE in formulation 17 were more released than formulations 8 and 18, respectively, with difference at 0.05 level of significance at every time interval after 10 minutes in release study. This result was affected by percent of oil phase A in preparation. Formulation 7 and 17 contains 17

Table 25 Student 's t distribution test of hypothesis and significance for DS creams

Formula	% drug released			t-value	Significance test
7	12.77	11.07	13.62	5.1572	S
8	7.29	7.49	7.97		
9	7.91	7.44	7.58	4.3800	S
10	6.39	6.04	6.70		
7	12.77	11.07	13.62	5.1865	S
9	7.91	7.44	7.58		
8	7.29	7.49	7.97	3.5493	S
10	6.39	6.04	6.70		

Remark : degree of freedom = 4
t 0.95 = 2.13

Table 26 Student 's t distribution test of hypothesis and significance for DE creams

Formula	% drug released			t-value	Significance test
17	11.75	12.07	12.64	6.6542	S
18	8.22	6.40	6.72		
19	9.72	9.53	9.62	7.0157	S
20	7.52	6.95	7.80		
17	11.75	12.07	12.64	7.7660	S
19	9.72	9.53	9.62		
18	8.22	6.40	6.72	0.4121	NS
20	7.52	6.95	7.80		

Remark : degree of freedom = 4
t 0.95 = 2.13

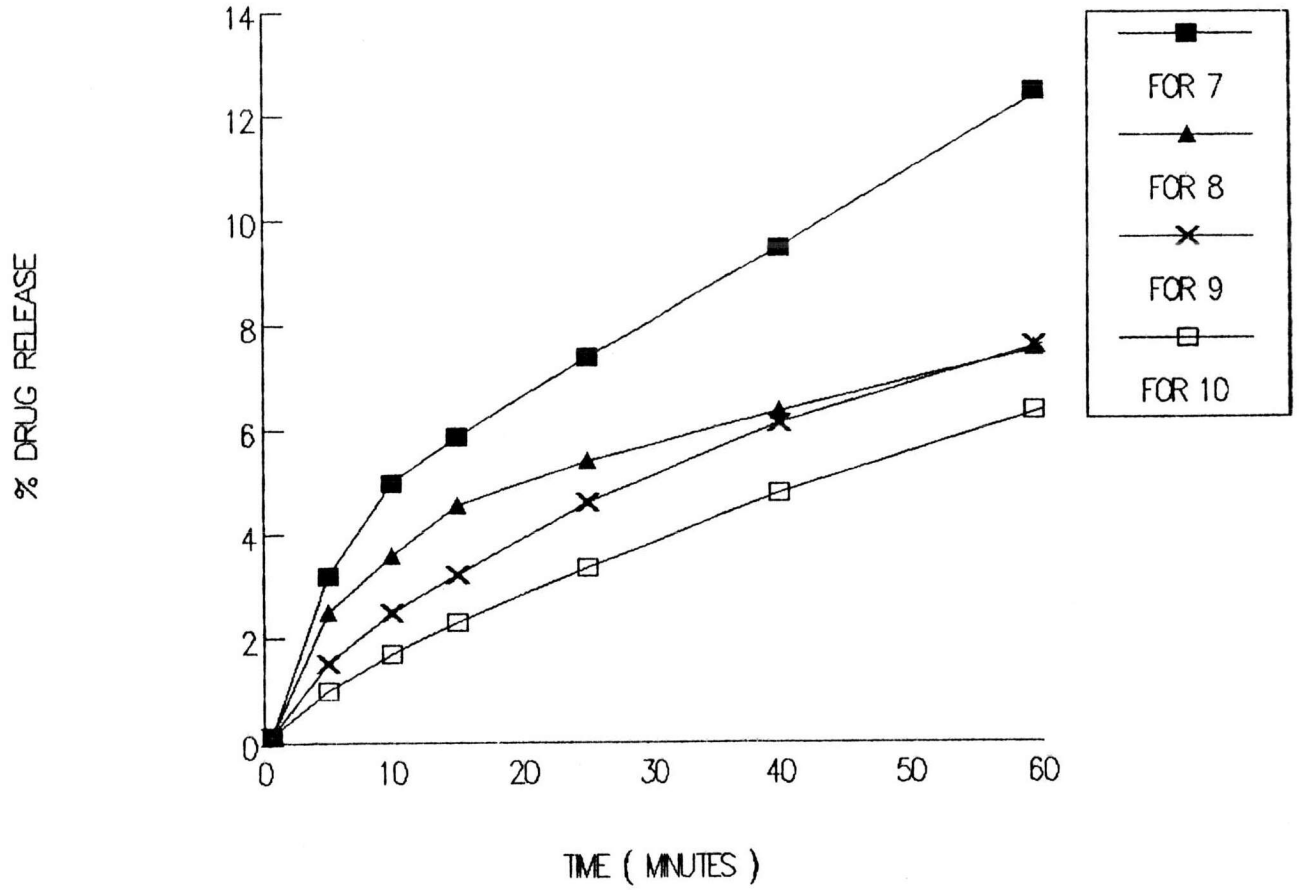


Figure 48 Plots of percent DS release from cream versus time of release study

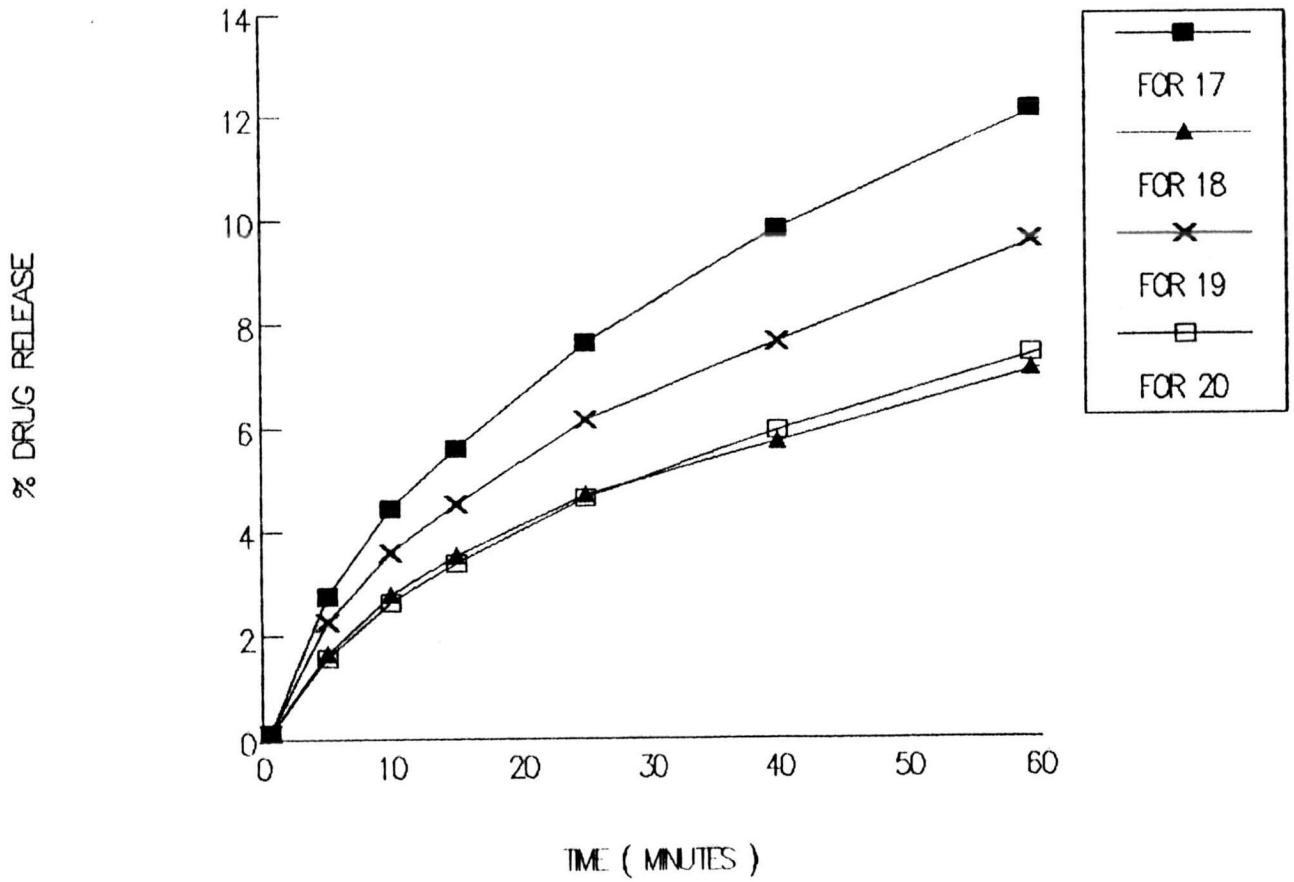


Figure 49 Plots of percent DE release from cream versus time of release study

percent of oil phase A wheseas formulation 8 and 18 contains 27 percent of oil phase A. Diclofenac in cream preparations were soluble in both oil and water phase. Diclofenac in water phase can directly diffuse or go around the oil globule through the membrane. Diclofenac which dissolved in oil golbules are firstly partition to the water phase before diffusion through the membrane in the same way as diclofenac in water phase. The increase of percent of oil phase results in increase amount of oil globule as well as diclofenac which dissolved in oil globules. So that an amount of released diclofenac are decrease. Similar explaination is to formulations 9, 10, 19 and 20.

1.2 Effect of Oil Phase Composition on Drug Release

A plot of percent drug release from cream which contains an equal amount of oil phase are shown in Figures 50 and 51.

DS in formulation 7 and DE in formulation 17 which contained 17 percent of oil phase A were more release than in formulations 9 and 19, respectively, which contains an equal percent of oil phase B with a difference at 0.05 level of significance at every time interval after 10 minutes in release study. This result may be affected by the difference of oil phase composition in these cream

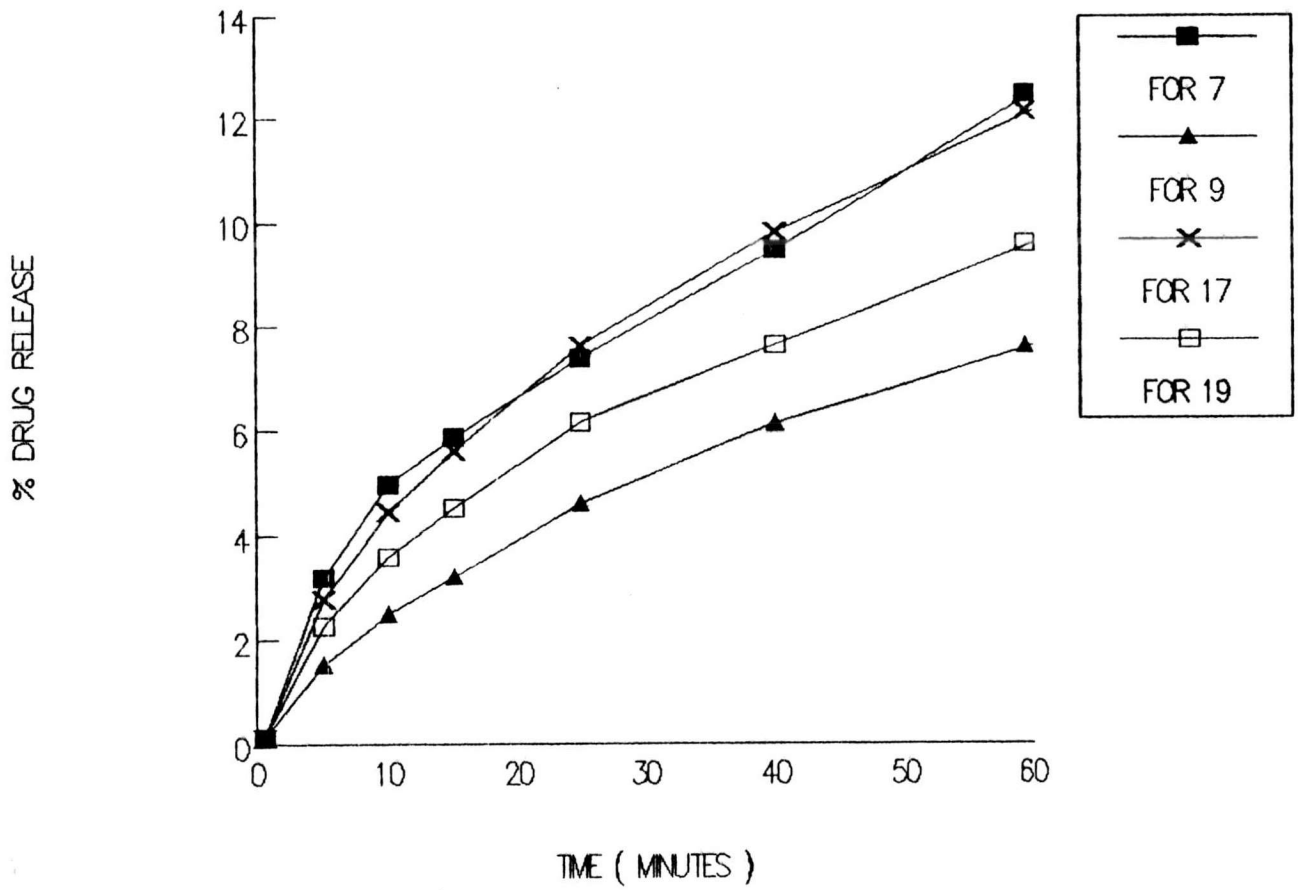


Figure 50 Plots of percent DS and DE release from creams which contained 17 percent of oil phase versus time of release study

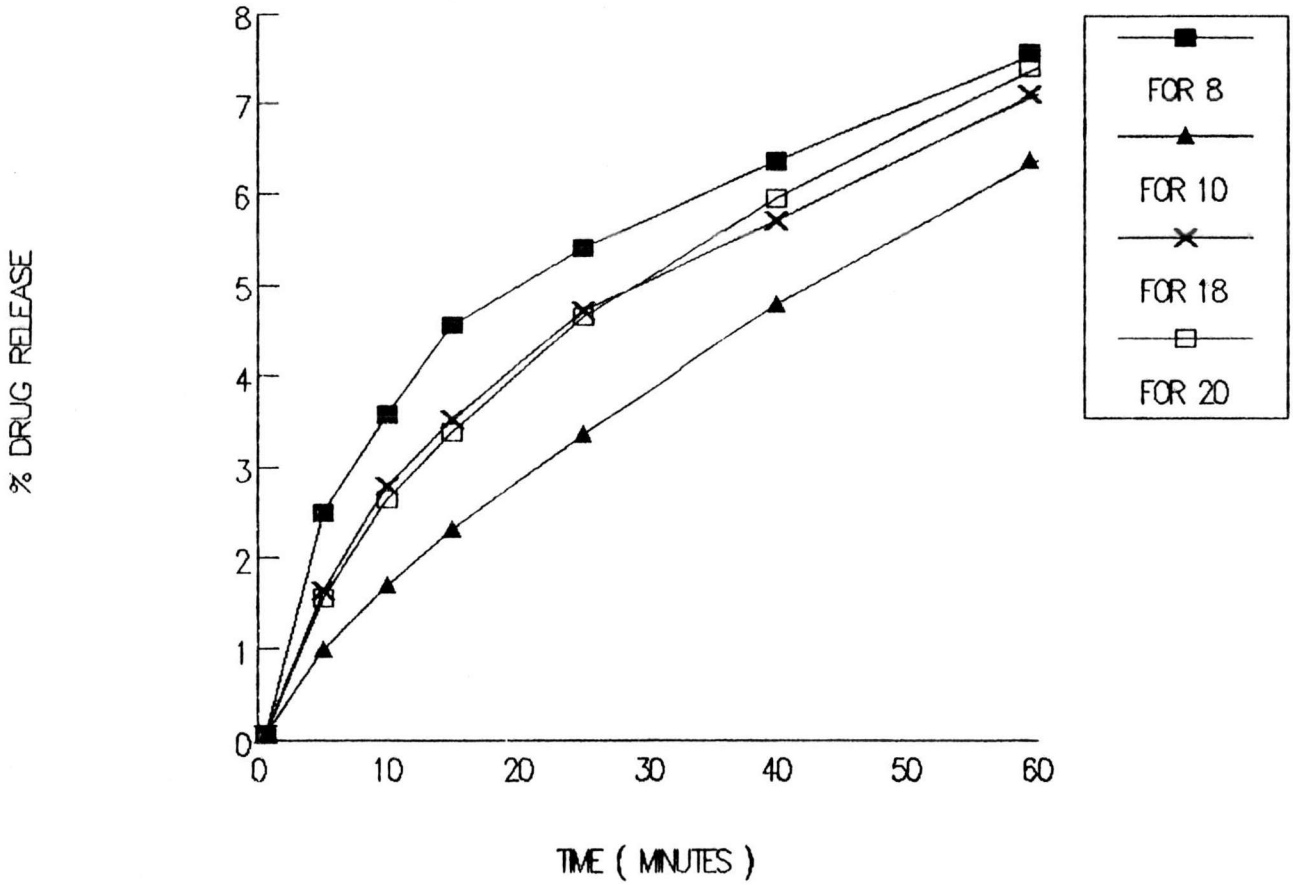


Figure 51 Plots of percent DS and DE release from creams which contained 27 percent of oil phase versus time of release study

preparations, thus, different solubility of DS and DE in oil phase. DS and DE are less soluble in oil phase A than oil phase B or more loosely bound to stearyl and cetyl alcohol than stearic acid, white beewax, and spermaceti. Therefore, DS and DE in oil phase A released easier release from cream than in oil phase B.

The same reason might be explained in 27 percent of oil phase cream, formulations 8 and 10, except for formulations 18 and 20 which amount of drug release is not different at a 0.05 level of significance. At 27 percent of oil phase, DE may be fully soluble in oil phase, so that oil composition did not affect the release of drug.

2. Hydrophilic Gels

A plot of percent DS and DE release from sodium CMC gel and poloxamer gel were illustrated in Figures 52 and 47, respectively. Student's t distribution test of hypothesis and significance of DS and DE hydrophilic gel were shown in Tables 27 and 28, respectively.

2.1 Effect of Percent of Gelling Agent in Hydrophilic Gel on Drug Release

DS and DE in formulations 53 and 59 which contained 1.5 percent of sodium CMC were more released than in formulations 54 and 60 which contained 2 percent of

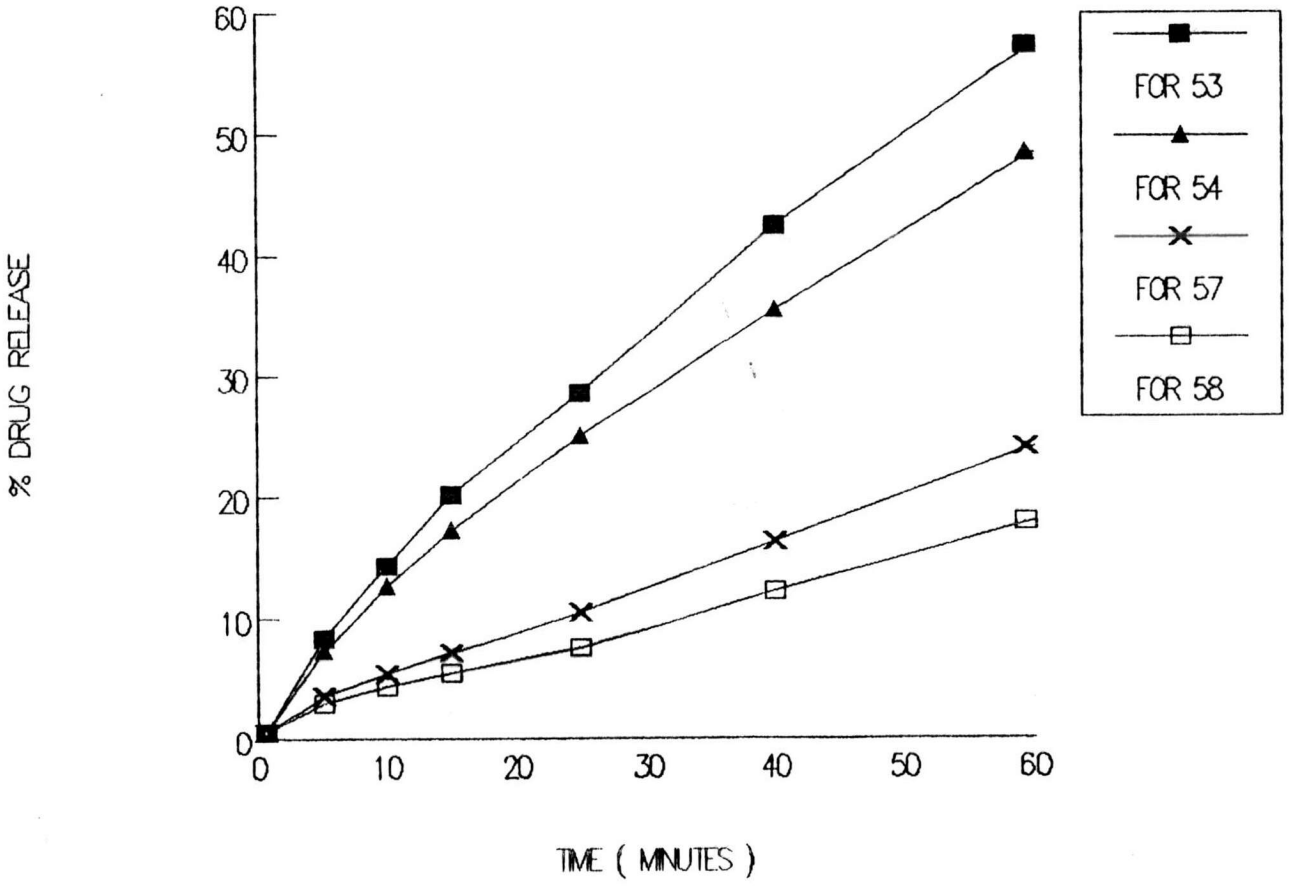


Figure 52 Plots of percent DS release from hydrophilic gels versus time of release study

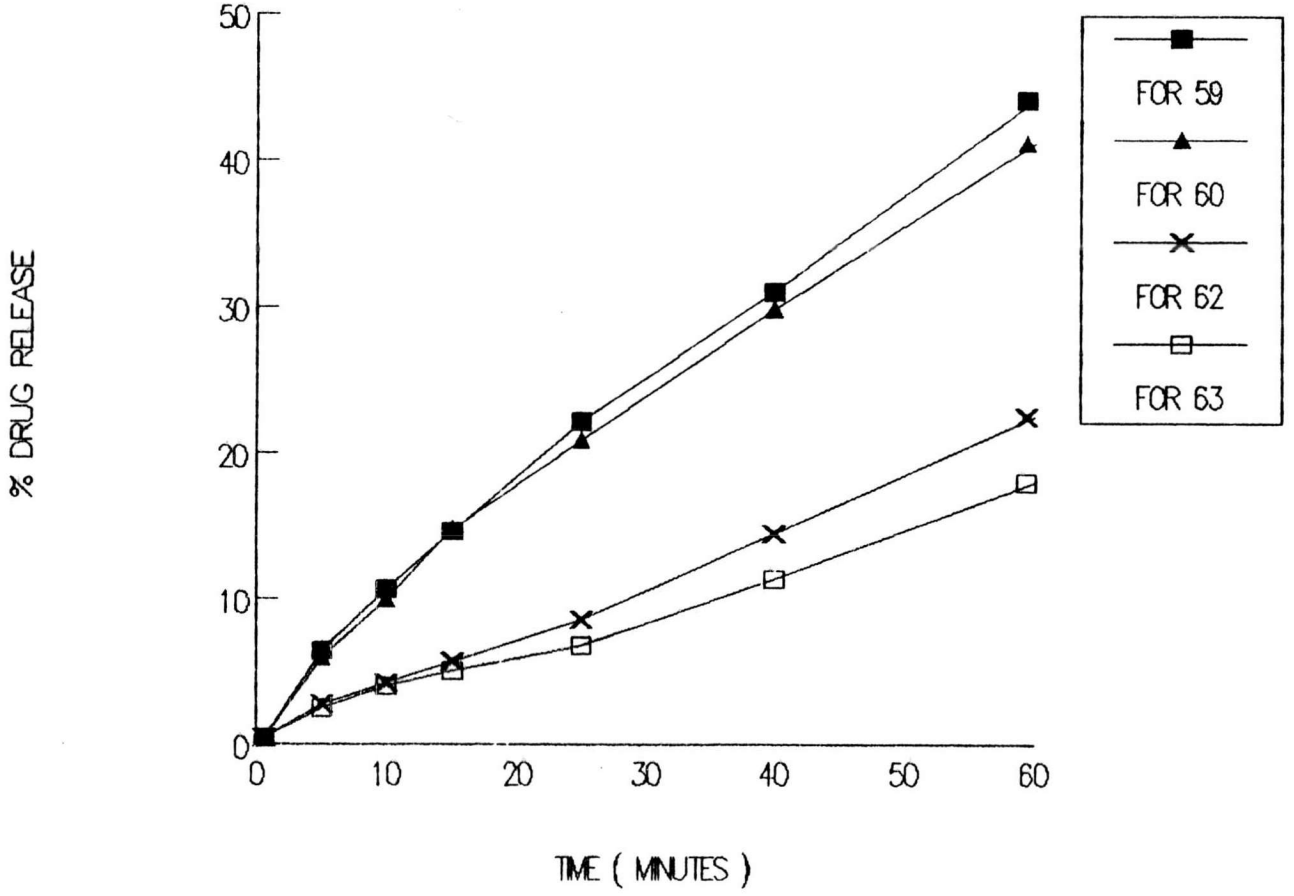


Figure 53 Plots of percent DE release from hydrophilic gels versus time of release study

Table 27 Student 's t distribution test of hypothesis and significance for DS hydrophilic gels

Formula	% drug released			t-value	Significance test
53	62.51	56.61	53.12	2.5784	S
54	47.33	49.73	48.40		
57	24.19	22.82	25.49	3.0367	S
58	20.94	16.45	16.66		
54	47.33	49.73	48.40	19.1423	S
57	24.19	22.82	25.49		

Remark : degree of freedom = 4
t 0.95 = 2.13

Table 28 Student 's t distribution test of hypothesis and significance for DE hydrophilic gels

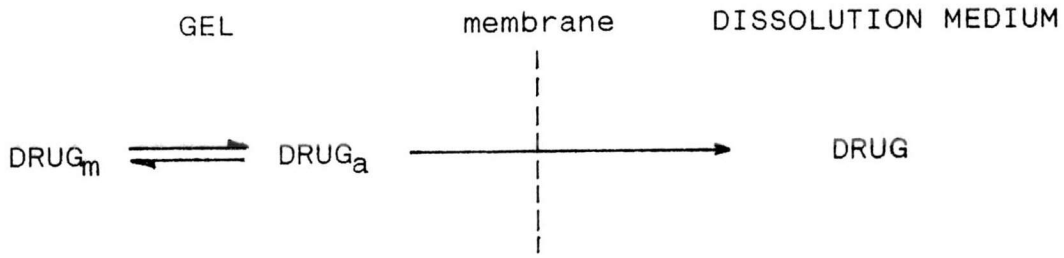
Formula	% drug released			t-value	Significance test
59	45.94	44.73	46.56	4.4888	S
60	40.20	41.11	42.35		
62	23.85	21.07	22.83	2.7872	S
63	20.15	16.98	17.00		
60	40.20	41.11	42.35	14.8679	S
62	23.85	21.07	22.83		

Remark : degree of freedom = 4
t 0.95 = 2.13

sodium CMC, respectively, with difference at 0.05 level of significance after 1 hour in release study. These results may affected by percent of sodium CMC in formulation. The more percent of gelling agent, the more viscosity of the gel that obstruct diffusion of drug from gel base through the membrane following Stokes-Einstein equation as preveous mentioned. An increasing amount of vehicle's as well as viscosity of gel will decrease the drug diffusion coefficient, that cause more drug release from 1.5 percent gel than from 2 percent gel after 1 hour of release study.

DS and DE in formulations 57 and 62 which contained 25 percent of poloxamer were more released than in formulations 58 and 63 which contained 30 percent of poloxamer, respectively, with difference at 0.05 level of significance at every time interval after 25 minutes in release study. These results may be affected by percent of poloxamer in formulation. Poloxamer is a gelling agent with nonionic surfactant and solubilizer properties which from micelle in medium (Tomida et al.,1987). Diclofenac can partially be solubilized into these micelle. Diclofenac which dissolved in water phase can directly diffuse through the membrane, whereas diclofenac which dissolved in micelle the same as oil globule in cream must firstly partition to water phase before diffuse through the membrane. Thus, the distribution equilibria of diclofenac in the gels and the release machanism through the

membrane may be written according to the following schem: where the subscripts a and m refer to the aqueous and micella phase, respectively.



An increase of percent of poloxamer leads to more viscous of gel and more micelle in gel as well as more solubilized drug in micelle , that causes decrease in diclofenac diffusion.

2.2 Effect of Type of Gelling Agent in Hydrophilic Gel on Drug Release

DS and DE were released from sodium CMC gel more than from poloxamer gel. Drug in sodium CMC gel dissolved in water phase between polymer chain, so that drug can directly diffuse through the membrane. On the other hand, drug in poloxamer gel, especially in micelle, must diffuse to water phase before go to the membrane, that cause lower amount of released drug than in sodium CMC gel.

3. Oil-Water Gels

Effect of Percent Oil Phase in Oil-Water Gels

A plot of percent of DS and DE released versus time of release study were shown in Figures 54 and 55, respectively. The release of drug from preparations which contained 0, 4 and 8 percent of oil were different at a 0.05 level of significance after 3, 4, 5 and 6 hours. An analysis of variance and the F test for the null hypothesis of equal mean of DS and DE preparation were shown in Tables 29 and 30, respectively.

The release of DS from preparations 79 and 80 which contained 0 and 4 percent of castor oil, respectively,were not different at a 0.05 level of significance. The release of DS from preparation 80 was not significantly more than preparation 81 which contained 8 percent of castor oil. But the release of DS from preparation 79 were significantly more than preparation 81 after 3 hours of release study. The increasing of castor oil from 0 to 8 percent reduced the release of DS from oil-water preparation.

The release of DE from preparation 89 which contained 0 percent of castor oil were not significantly more than preparation 90 which contained 4 percent of castor oil. But the release of DE from preparations 89 and 90 were more than preparation 91 which contained 8 percent

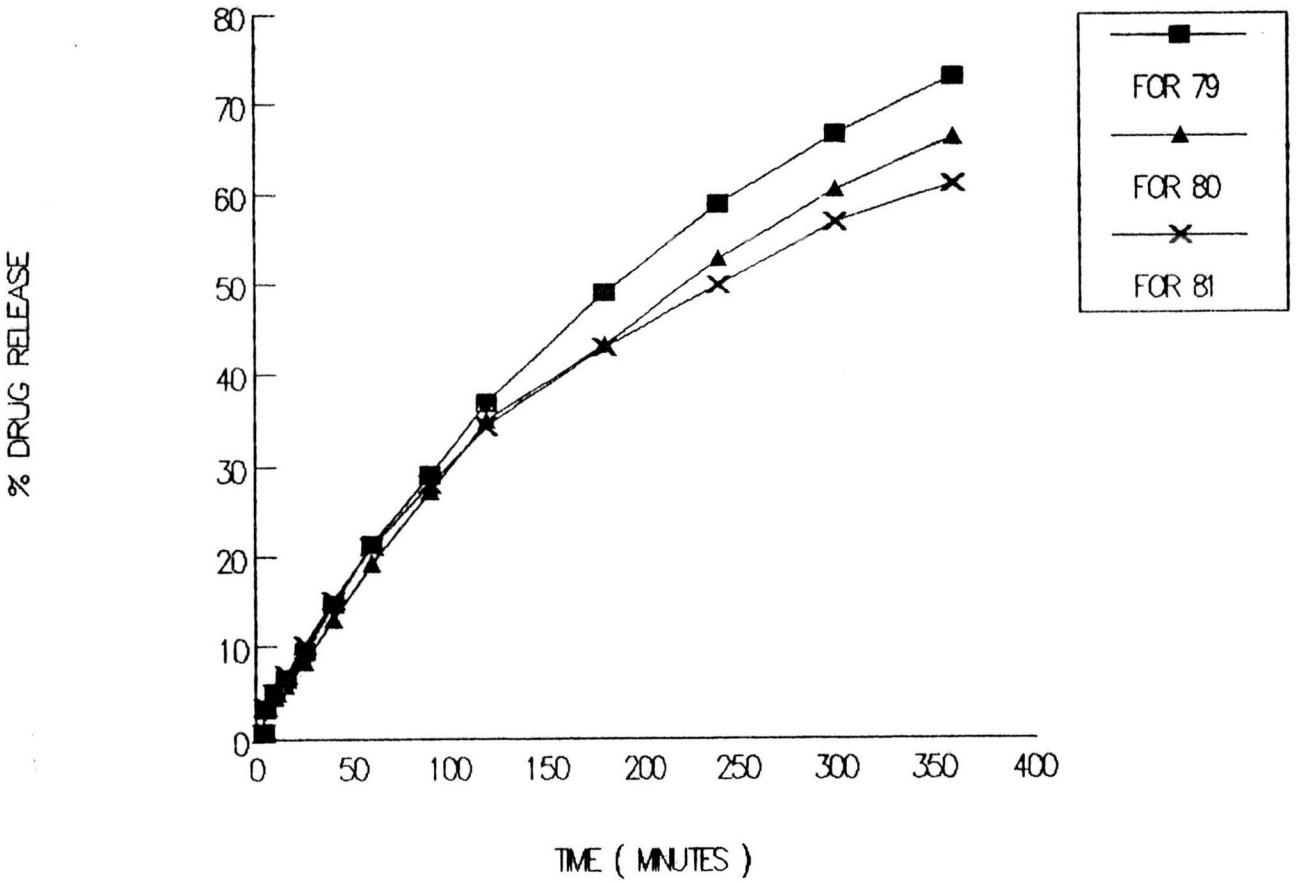


Figure 54 Plots of percent DS release from oil-water gels versus time of release study

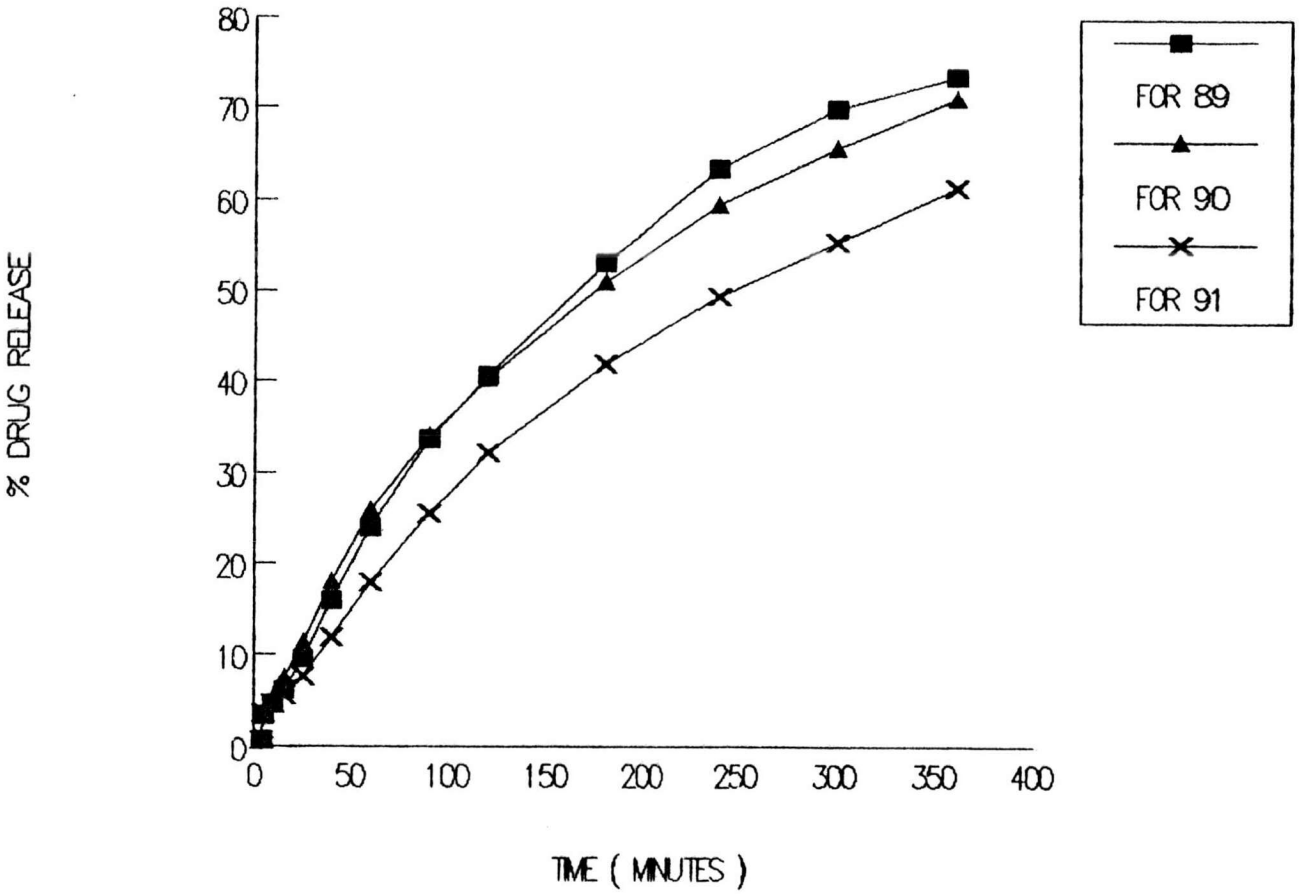


Figure 55 Plots of percent DE release from oil-water gels versus time of release study

Table 29 Analysis of variance and the F test for the null hypothesis of equal means , DS Oil-Water gels

Time (hours)	% drug released			F-value	Significance test
	79	80	81		
1	24.30	25.23	22.60	0.3151	NS
	19.50	16.19	18.48		
	20.25	16.23	22.27		
2	41.22	43.60	35.50	0.2174	NS
	35.56	29.92	31.72		
	34.00	31.10	35.82		
3	52.40	45.91	43.50	5.5238	S
	47.55	39.75	41.74		
	47.39	44.14	43.77		
4	62.13	55.65	50.11	7.6849	S
	60.31	50.01	49.63		
	54.27	52.74	49.78		
5	68.92	61.91	57.11	10.1981	S
	69.29	58.08	57.98		
	62.13	61.43	55.63		
6	74.95	66.04	60.30	10.4375	S
	76.02	64.14	64.05		
	68.21	69.04	59.27		

Remark : degree of freedom = 2,6
F 0.95 = 5.14

Table 30 Analysis of variance and the F test for the null hypothesis of equal means , DE Oil-Water gels

Time (hours)	% drug released			F-value	Significance test
	89	90	91		
1	26.10	26.64	20.62	11.9212	S
	21.42	25.86	17.74		
	24.34	24.91	15.31		
2	45.63	40.90	35.53	4.3644	NS
	33.80	39.89	31.62		
	42.48	40.12	29.21		
3	57.90	53.70	45.46	7.9131	S
	47.96	50.56	40.62		
	53.53	48.87	39.47		
4	68.85	62.13	53.81	19.9895	S
	55.72	58.90	47.45		
		57.28	47.02		
5	76.31	67.62	59.90	7.2354	S
	62.25	63.77	53.37		
	71.26	65.37	52.84		
6	78.70	75.24	66.06	5.6568	S
	67.53	70.31	60.40		
	74.11	67.50	57.49		

Remark : degree of freedom = 2,6
F 0.95 = 5.14

of castor oil at a 0.05 level of significance after 3 hour in release study. The increasing of castor oil from 0 to 8 percent and from 4 to 8 percent reduced the release of DE from oil-water gel in the same manner.

These results may be affected by percent of oil phase in preparation.

Preparations which contained no oil phase are composed of micelle which formed by poloxamer and PHC. PHC is nonionic emulsifying and solubilizing agent. Drug can dissolved in both micella and water phase.

Preparations which contained 4 percent of oil phase are composed of micelle and oil which completely solubilized in micelle. Drug can dissolve in micelle, oil in micelle and water phase.

Preparations which contained 8 percent of oil phase are composed of micelle with solubilized oil and oil globule with adsorped monolayer of nonionic emulsifier. Drug can dissolve in micelle, oil in micelle, oil globule and water phase.

The same reason as increasing of oil phase in cream, The increase of oil phase in oil-water gel decreased the release of diclofenac.

4. Comparision of the Preparations with Commercial Products

A plot of percent drug release from three commercial product versus time of storage was shown in Figure 56.

Formulation 7, 53, 57 and 79 were chosed as they showed the most DS released from cream, sodium CMC gel, poloxamer gel and oil-water gel, respectively. Product C was used as a model DS topical preparation. A plot of percent drug release versus time of study of formulation 7, 53, 57, 79 and product C was shown in Figure 57. The general rank significant order of drug release was : formulation 53 > product C > formulation 57 > formulation 79 > formulation 7. Product C was clear gel as sodium CMC gel and poloxamer gel. The release of DS from product C was more than from poloxamer gel but less than from sodium CMC gel.

Formulations 17, 59, 62 and 89 were choosed as they showed the most DE released from cream, sodium CMC gel, poloxamer gel and oil-water gel, respectively. Product A was used as a model DE topical preparation. A plot of percent drug release versus time of study of formulation 17, 59, 62, 89 and product A was shown in Figure 58. The general rank significant order of drug release was : formulation 59 > product A > formulation 89 > formulation 62 > formulation 17.

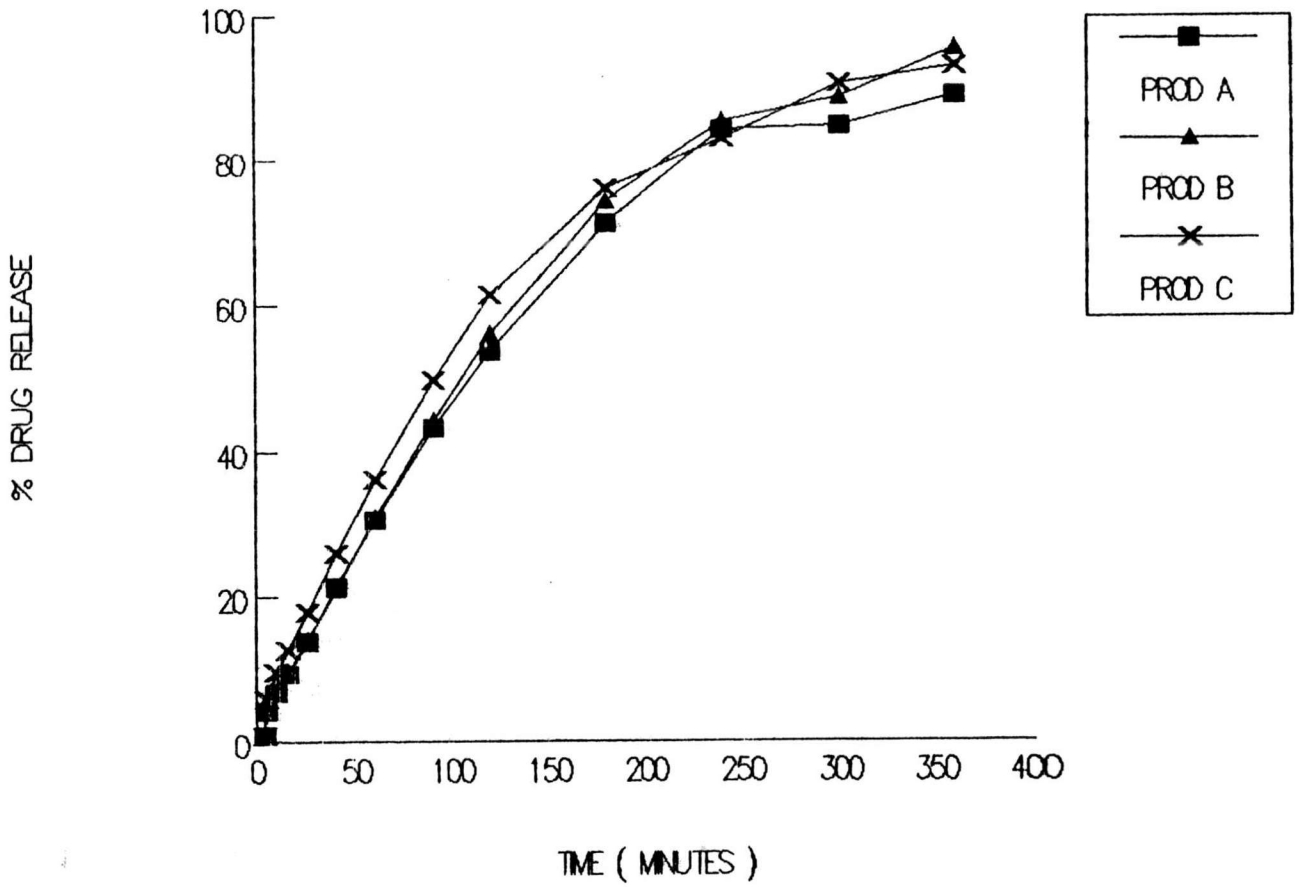


Figure 56 Plots of percent diclofenac release from commercial products

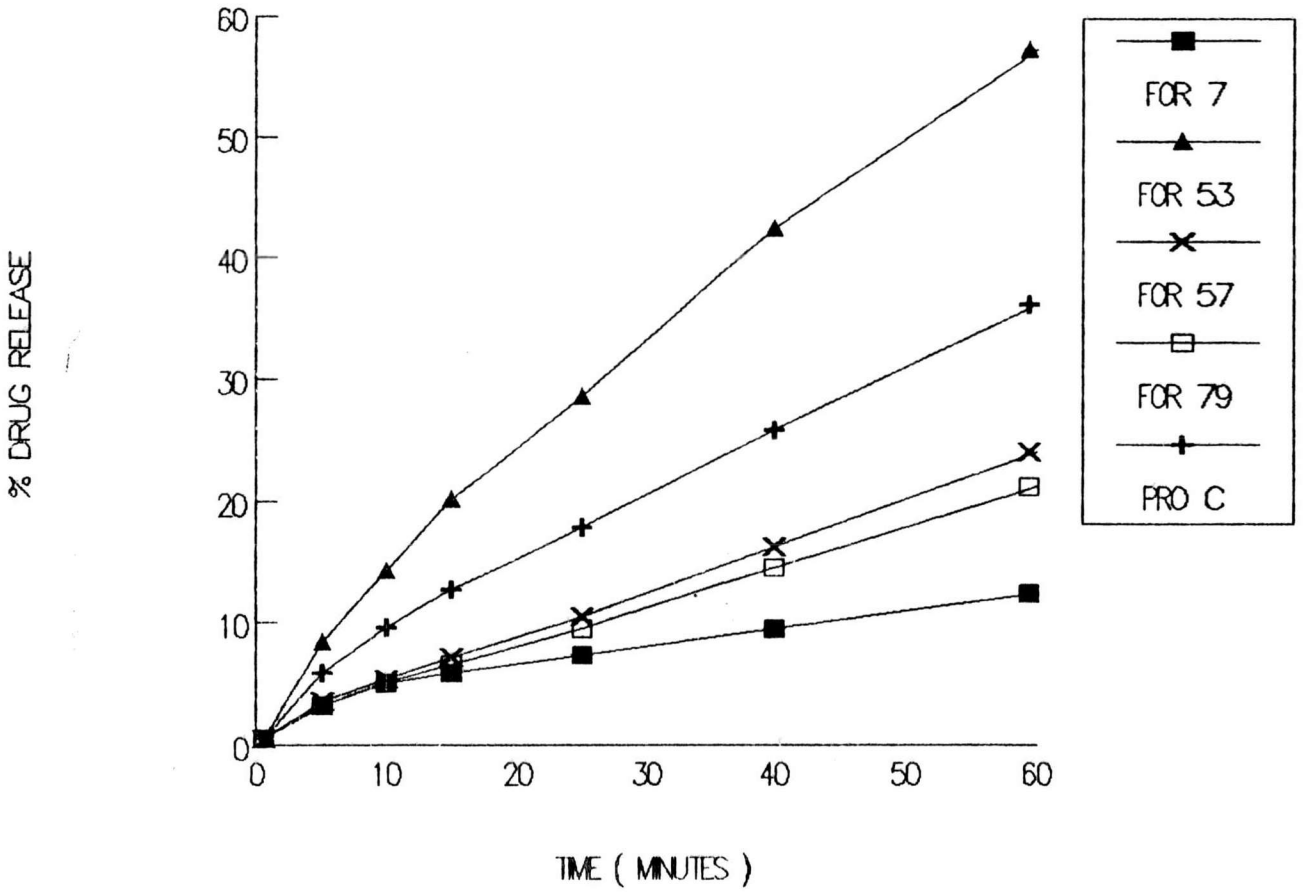


Figure 57 Plots of percent DS release from formulation 7, 53, 57, 79 and product C versus time of release study

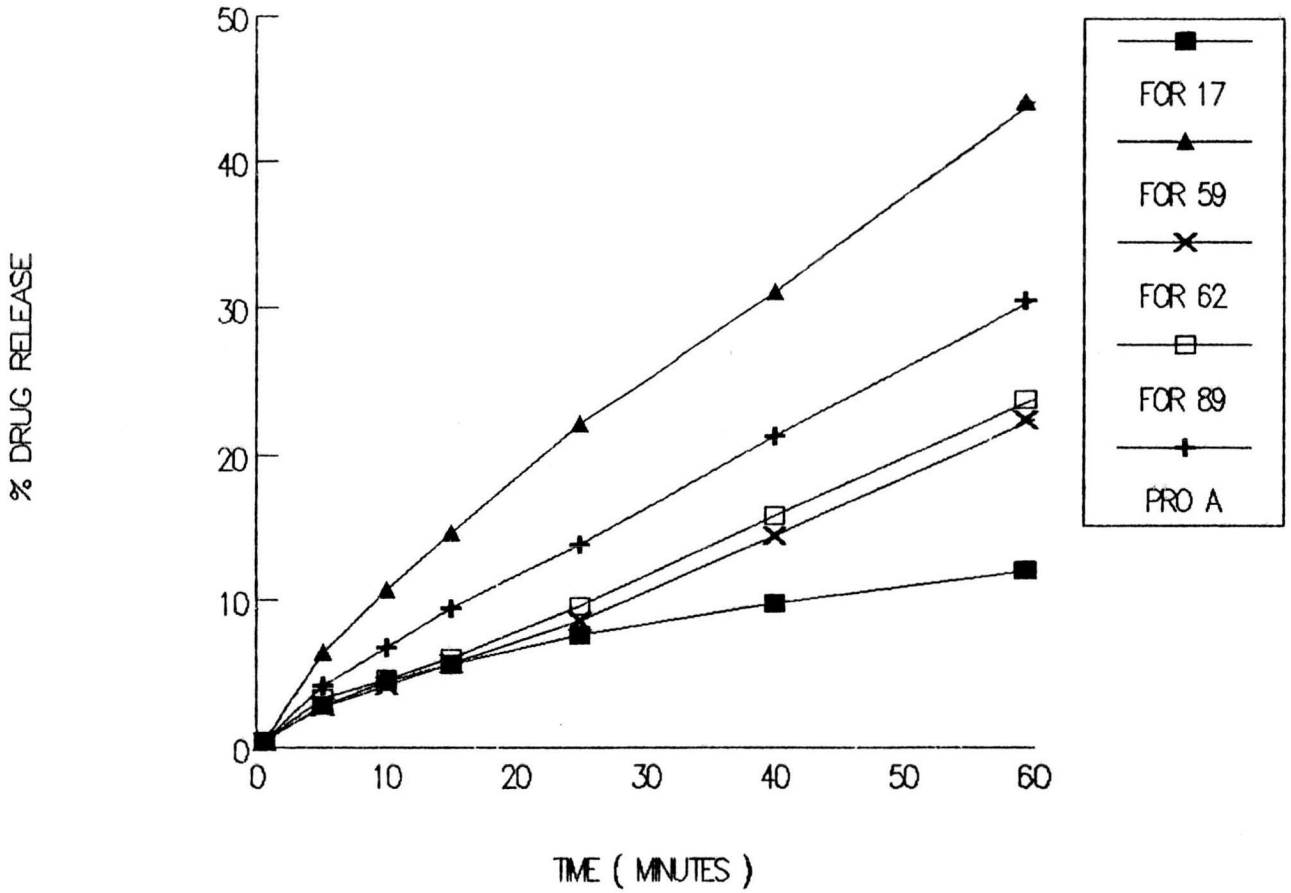


Figure 58 Plots of percent DE release from formulation 17, 59, 62, 89 and product A versus time of release study

5. Effect of Diclofenac Salts on Drug Released

The effect of diclofenac salts on drug release is depended on the vehicle or ingredient in the formulation. For cream containing mineral oil, stearyl alcohol, and cetyl alcohol (oil phase A) , poloxamer gels and oil-water gels, either type of diclofenac salts produced the same release of drug, whereas for creams containing mineral oil, stearic acid, white beewax, stearyl alcohol and spermaceti (oil phase B), DS is less released than DE and for sodium CMC gel, DS is conversely released more than DE (α = 0.05). Student 's t distribution test of hypothesis and significance for DS and DE preparations are shown in Table 31.

In both 17 and 27 percent of oil phase B, DS was less released than DE (Figure 59). These results may be affected by the different solubility of diclofenac salts in these oil phase. DS may be more soluble in these oil phase than DE so that the percentage of DS which partition to water phase is less than DE, that cause the less percentage of DS released than DE in these cream preparation which contains equal amount of oil phase.

DS was released more than DE from preparation which contained an equal percent of sodium CMC (Figure 60). These results may be affected by the different diclofenac salts and sodium CMC interaction. DS may be more loosely bound to

Table 31 Student 's t distribution test of hypothesis and significnace for diclofenac sodium and diclofenac diethylammonium preparations.

Formula	Time (hr.)	Mean of % Drug release	t-value	Significance test
7,17	1	12.49,12.15	0.3529	NS
8,18	1	7.58, 7.12	0.6437	NS
9,19	1	7.64, 9.62	10.7964	S
10,20	1	6.38, 7.42	2.7176	S
53,59	1	57.41,45.74	3.4122	S
54,60	1	48.48,41.22	6.3607	S
57,62	1	24.17,22.58	1.1547	NS
58,63	1	18.02,18.04	0.0121	NS
79,89	1	21.35,23.95	1.0517	NS
80,90	1	19.22,25.80	1.7644	NS
81,91	1	21.12,17.89	1.3007	NS
79,89	6	73.06,73.45	0.0778	NS
80,90	6	66.40,71.02	1.4093	NS
81,91	6	61.21,61.31	0.0309	NS

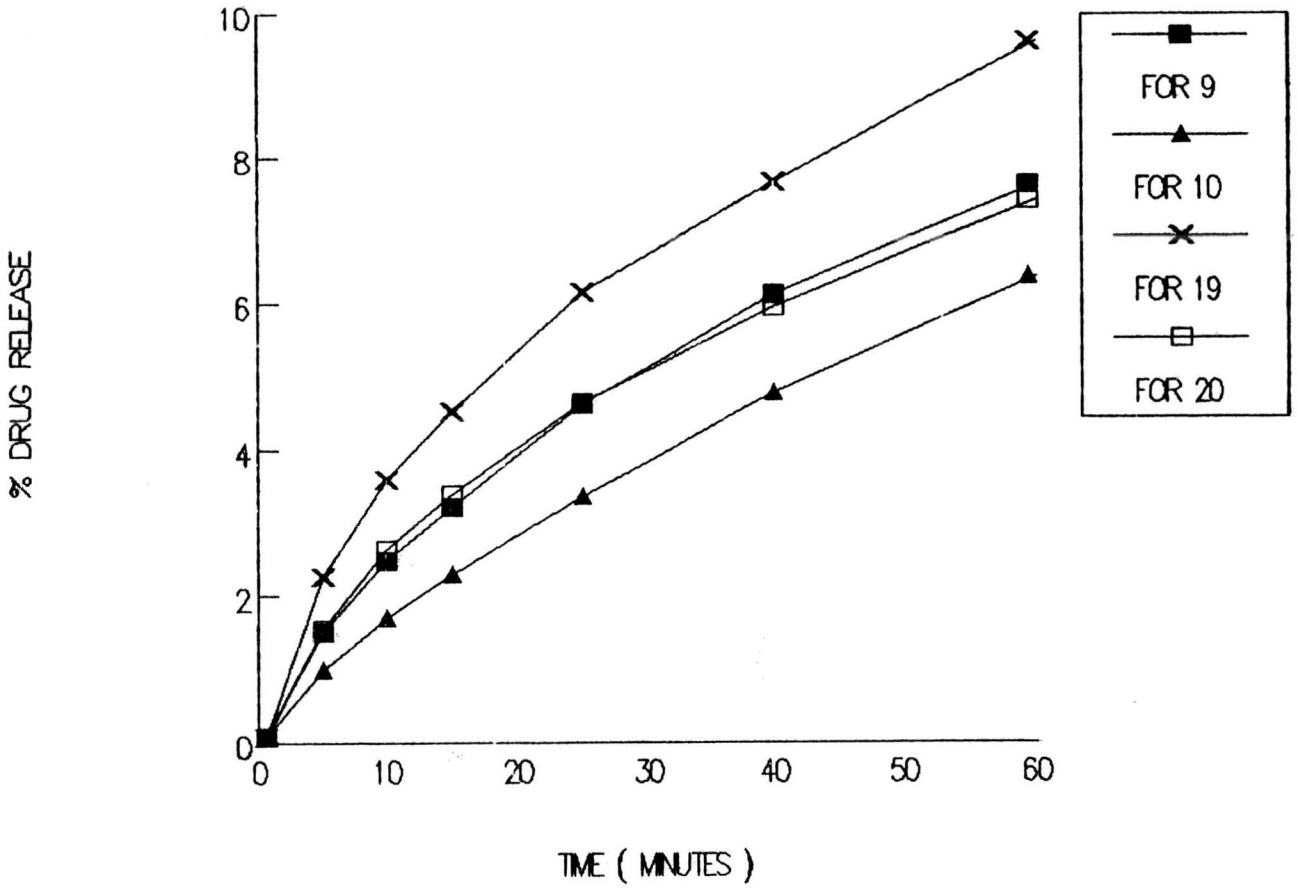


Figure 59 Plots of percent release of DS and DE from oil phase B cream

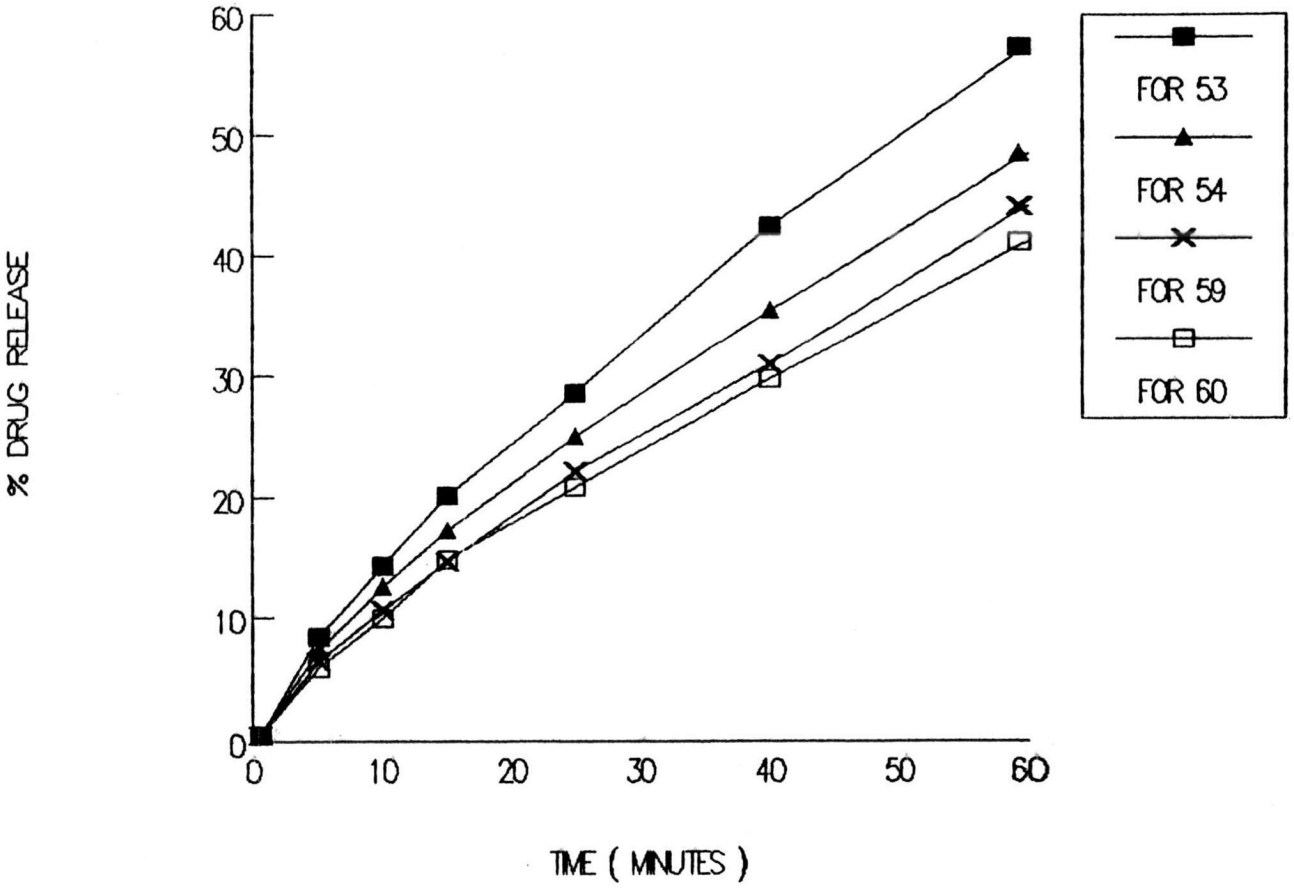


Figure 60 Plots of percent release of DS and DE from sodium CMC gel

sodium CMC polymer chain than DE. Another reason is, DS may be repulsed by sodium ion of sodium CMC more than DE. These reasons caused more diffusion of DS than that of DE in these preparation.

Conclusion

Salt forms of diclofenac affected the physicochemical such as crystal forms and shape of crystal in scanning electron microscope, IR spectra, solubility, stability and the release of drug from preparation especially in cream (oil phase B) which contained mineral oil, stearic acid, white beeswax, stearyl alcohol and spermaceti as an oil phase and sodium CMC gel. DS released significantly less than DE in the former cream but more than DE in sodium CMC gel. These results may indicated that DS was less soluble than DE in oil phase B. The repulsive force between DS and the polar group of sodium CMC chain was more than that caused by DE. The solubility of diclofenac in water were increasing as the increase of pH as well as increasing of ionized form of molecule. The general rank order of diclofenac 's solubility in solvent was: methanol > propylene glycol > ethanol > glycerin > isopropyl alcohol > sorbitol solution, depended on solvent's carbon and hydroxyl group ratio and solvent 's viscosity. Diclofenac in pH 6, 7, 8 and 9 solution were similarly stable. More than 90 percent of drug was remained after 5 months of storage at 35 ^{o}C. The stability of diclofenac increased as well as the increase of buffer pH. At the same pH diclofenac was rapidly degraded at the higher temperature of storage following Arrhenius equation. The stability of diclofenac in pH 5 solution is the least correlated to minimum energy of activation which calculated from

Arrhenius plot. Emulsifier that was suitable for using in cream preparation was cetomacrogol 1000 which gave good appearance and no segregation after freeze-thaw cycles on the other hand Tween and Span produced the opposite physical appearance. Oil phase composition of cream significantly affected on the release of diclofenac. Oil phaseA cream (mineral oil, stearyl alcohol and cetyl alcohol) was softener and low viscosity than group B cream so that diclofenac was more release from oil phase A cream than oil phase B cream. Percent of oil phase in cream and oil-water gel significantly affected on the release of drug. The increase of oil phase induced more soluble of drug in oil phase thus decreased the release of drug from preparation. The same as increase of oil phase in the previous praparation, the increase of amount of poloxamer as well as micelle and drug which soluble in micelle in poloxamer gel formulation reduced the release of drug. Increasing percent of sodium CMC as well as viscosity of preparation in sodium CMC gel significantly decreased the percent of drug release. The release of drug from sodium CMC gel was significantly more than from poloxamer gel. These results may be affected by poloxamer micelle in which drug could be solubilized whereas no micelle was obtained from sodium CMC. So that drug in sodium CMC gel was easily diffuse through the membrane. On comparision of preparation with commercial product, the general rank order of drug release was: sodium CMC gel > commercial product > oil-water gel ~ poloxamer gel > cream.

Suggestion in the Future Study.

In further study, formulation 7,17,53,57,59,62,79,89, product A, B and C should be tested for drug and preparation stability at several temperatures and conditions, then in-vivo tested in nude mice, and later in healthy subject. Amount of drug absorbed should be plotted and compares with in-vitro data. The relationship between in-vivo study and in-vitro data sould be evaluated. If the relation is attained, The conversion factor that transformed data from in-vitro data to in-vivo could be searched and established. Therefore, in the later study, only in-vitro data are able to employ for prediction the in-vivo profile, that could save time and cost of evaluation. The relation between in-vivo and in-vitro data may be used to develop in-vitro release method, diffusion cell and membrane in order to decrease the difference between in-vivo and in-vitro data. So that we can predicted a closely correct in-vivo data from in-vitro data. Lastly, only in-vitro study can use to formulate the optimum topical preparation with good bioavialability.