CHAPTER III

RESULTS AND DISCUSSION

An objective of this study is to study the effect of various enhancers on diclofenac sodium flux through human placental membrane, human amnion and newborn pig skin. Jittida (1994) had compared steady state fluxes of certain NSAIDS (piroxicam, phenylbutazone, indomethacin, diclofenac diethylammonium, and diclofenac sodium) through cellulose acetate - silastic® - cellulose acetate membranes, human placental membrane, human amnion, and newborn pig skin. The results showed that the general rank orders of steady state fluxes of the five NSAIDS through newborn pig skin, human amnion and human placental membrane were the same, that was: piroxicam < phenylbutazone < indomethacin < diclofenac diethylammonium < diclofenac sodium. However, the rank order of steady state fluxes through the synthetic membrane was different from the others. Therefore, human amnion and human placental membrane might replace newborn pig skin for flux comparisons of the five NSAIDS from formulations not containing any additives affecting the membrane. A further study is therefore needed to examine whether a chemical altering the membranes' barrier (i.e., an enhancer) will have a similar effect on the steady state fluxes through the three biological membranes. Diclofenac sodium was chosen as a model drug in this study because it gave the highest flux through the three membranes.

For donor solutions, saturated diclofenac sodium solutions had been used in part of this study to obtain a constant activity. This can be hopefully assumed that the enhancing effect was resulted only from enhancers used. The receiving solution is usually aqueous systems and should not alter the barrier properties of the membrane except from the hydration effect. A prime objective of receptor fluid is to provide a sink condition for penetrating molecules during the diffusion studies. It is generally accepted that the sink condition is maintained when the concentration of penetrant in the receiving solution does not exceed 5% of the donor concentration (Daynes, 1920).

In this study, the analysis of drug contents in the receptor solution would be done after one day of the *in vitro* diffusion experiments. Samples had been kept in a freezer before the analysis were done. The chemical stability of drug in receiving solution at 37 ± 1°C had been studied for seven days and there was no sign of degradation (Jittida, 1994). No peak of degradation product had been found using the HPLC method.

1. Solubility of Diclofenac Sodium in Donor Solutions.

The solubility of diclofenac sodium in donor solutions at 33 ± 1°C is shown in Table 1 and Appendix II. The temperature of 33°C was chosen since it is an average temperature of this laboratory room. The equilibrium time of saturated solutions was at least 48 hours. The least solubility of diclofenac sodium is observed in isopropanol and the highest solubility is in 10% w/v tetraglycol. Diclofenac sodium is a salt so it should dissolve well in water. However, the value of partition coefficient of diclofenac sodium in n-octanol / pH 7.4 aqueous buffer which is 13.4

Table 1: Solubitity of diclofenac sodium in donor solutions at 33 ± 1°C.

Donor vehicle	Solubility * (mg/ml)
Water	71.67 ± 0.006
0.01 mg/ml Tween 20 in water	92.78 ± 1.292
0.05 mg/ml Tween 20 in water	94.53 ± 1.231
0.4 % w/v Brij 35 in water	102.72 ± 0.007
1 % w/v Brij 35 in water	126.47 ± 0.011
10 % w/v Propylene glycol in water	274.10 ± 0.018
10 % w/v Tetraglycol in water	360.52 ± 0.032
Ethanol	330.84 ± 0.025
Isopropyl alcohol	40.26 ± 0.003
1 % Orange oil in ethanol	352.53 ± 0.003

* n = 6, mean ± SD.

(Adeyeye and Pui-Kui, 1990) indicates that diclofenac sodium is lipophilic. So it is more soluble in glycols (propylene glycol and tetraglycol) and ethanolic solutions (ethanol and 1% w/v orange oil in ethanol). Because the solubility parameters of isopropanol, ethanol and propylene glycol are 12.0, 13.0, and 14.8, respectively, the solubility parameter of diclofenac sodium should be closer to ethanol and the glycol solutions. Therefore, the drug solubility in isopropanol is less.

For the solubility determination, a great care must be taken due to the ease of evaporation of ethanol and isopropanol. The coefficient of variation (%CV) of the six solubility values obtained must be within 2-3%.

2. Partition Coefficients of Diclofenac Sodium between Certain Donor Vehicles and the Membranes.

The penetration of a drug molecule involves the movement of drug from a vehicle to a barrier membrane. The ability of drug to escape from the vehicle is measured by partition coefficient (K) which is defined as the equilibrium solubility of drug in the surface of the membrane relative to its solubility in the vehicle:

$$K = \frac{\text{Drug equilibrium concentration (membrane)}}{\text{Drug equilibrium concentration (vehicle)}} \qquad (10)$$

Partition coefficient data between the three membranes and saturated donor solutions are shown in Table 2. If the solubility of drug in a particular membrane is constant, an increase in drug solubility in the vehicles should lead to a decrease in the K value. The results are not what expected. This should be due to

Table2: Partition coefficient data between certain donor vehicles and the three membranes.

Donor vehicle	Pig skin		Human amnion		Human placental membrane	
	K *	%CV	K*	%CV	K *	%CV
Water	6.97 ± 3.11	44.62	132.32 ± 42.66	32.24	18.08 ± 17.92	99.11
0.05 mg/ml Tween 20 in water	8.74 ± 3.97	45.38	15.88 ± 13.66	85.98	28.38 ± 14.90	52.50
1.0 % w/v Brij 35 in water	11.34 ± 4.93	43.28	70.43 ± 6.51	33.48	21.30 ± 11.97	84.37
10 % w/v Propylene glycol in water	39.58 ± 14.46	36.53	41.30 ± 24.71	60.22	55.99 ± 11.97	21.30
10 % w/v Tetraglycol in water	24.82 ± 7.11	28.53	90.47 ± 16.59	18.34	42.79 ± 16.51	38.50

* n = 3, mean ± SD.

the fact that the vehicles must affect the membrane differently and therefore the drug solubility in the membrane is not constant but it is dependent upon the vehicle studied.

The partition coefficient values of diclofenac sodium between the vehicles and pig skin are lower than those between the vehicles and human amnion and placental membrane. This may result from the differences in the structure and composition of the three membranes. Pig skin has some hair which may obstruct the partition of drug into the skin whereas surfaces of amnion and placental membrane are smooth and hairless. Lipid contents in the three membranes that impede the penetration of drug are also different. Since human amnion and human placental membrane have partition coefficient values that are greater than those of pig skin, diclofenac sodium should penetrate through human amnion and human placental membrane faster than through pig skin as the partition coefficient controls the concentration gradient across the membrane and thus the penetration rate.

3. *In Vitro* Diffusion Studies.

It is clear that a mechanism of drug transport across the skin is essentially a passive diffusion. This is a phenomenon by which a diffusant moves down a concentration gradient (or more accurately, a chemical potential gradient) by random molecular motion. In the situation of a permeant entering the skin, the percutaneous absorption is considered to be unidirectional, i.e., the concentration gradient is directed into the skin as described by Fick's first law (Eq. 5).

Fick's first law states that the permeation rate of drug depends upon concentration gradient, diffusion coefficient, partition coefficient, and membrane thickness. Anything that effect these parameters results in the change of permeation rate.

Data of individual diffusion run are given in Appendix III through V. The average normalized fluxes were utilized to compare the permeability of drugs through various membranes. The end of sampling time in the cases of human amnion and placental membrane (80 minutes) was shorter than that in the case of pig skin (160 minutes) because the drug could permeate through human amnion and human placental membrane much quicker than through newborn pig skin. Times to reach steady state diffusion were also shorter in the cases of human amnion and placental membrane.

The coefficient of variations (% CV) of the steady state fluxes across newborn pig skin, human amnion, and human placental membrane are 2.780-99.16%, 2.626-42.90% and 5.197-56.89%, respectively. Therefore, the permeability data using pig skin are less reproducible than those using human amnion and placental membrane.

3.1 25 mg/ml of Diclofenac Sodium Solution as a Donor Phase.

While the permeation through skin is a passive process, the primary barrier of the skin, the stratum corneum, is not inert. Interactions of drugs, solvent, and other substances with the stratum corneum may lead to changes in its resistance

to diffusional transfer. In general, alterations in vehicle formulations affect one or both of the following: escaping tendency of drug from the vehicle and skin membrane resistance. Ideally, saturated solutions of the same drug in different solvents should have the same escaping tendency. In the saturated solutions, a dissolved drug is in equilibrium with its excess solid whose a value of thermodynamic activity is assigned to be unity. The activity of the dissolved drug is also unity. At a lower drug concentration, the escaping tendency is reduced because of its lower activity. Since the flux of drug is proportional to drug concentration, the ratio of drug concentration to its solubility in the vehicle may be used as an indicator of escaping tendency. A change of vehicle to the one that is capable of increasing drug solubility leads to a decrease in escaping tendency which leads to a decrease in percutaneous absorption if the drug concentration in the solution is kept constant. In this case, the vehicles must not affect the membrane (Zatz and Pramod, 1987).

The steady state fluxes of 25 mg/ml diclofenac sodium in donor vehicles (water, 0.05 mg/ml tween 20 in water, 1.0% w/v brij 35 in water, 10% w/v propylene glycol in water and 10% w/v tetraglycol in water) through newborn pig skin, human amnion and human placental membrane are shown in Table 3-5. The correlations of fluxes and solubilities of diclofenac sodium are shown in Figure 11-12.

In the case of pig skin (Figure 10), the surfactants and glycols increase the solubility of diclofenac sodium but the fluxes of a fixed concentration do not decrease. This indicates that both surfactants and glycols are not inert. They must interact with pig skin. In the other words, they act as enhancers that decrease the diffusional resistance so the fluxes increase. The surfactants (0.05 mg/ml tween

Table 3: Fluxes of diclofenac sodium from its 25 mg/ml solution through newborn pig skin.

Donor vehicle	Steady state flux* (mcg/min cm^2)	% CV
Water	42.09 ± 1.17	2.78
0.05 mg/ml Tween 20 in water	186.10 ± 145.10	78.01
1 % w/v Brij 35 in water	320.03 ± 100.80	31.17
10 % w/v Propylene glycol in water	95.32 ± 36.83	38.64
10 % w/v Tetraglycol in water	47.88 ± 5.21	10.88

* n = 3, mean ± SD.

Table 4: Fluxes of diclofenac sodium from its 25 mg/ml solution through human amnion.

Donor vehicle	Steady state flux* (mg/min cm^2)	% CV
Water	334.33 ± 79.91	23.90
0.05 mg/ml Tween 20 in water	404.03 ± 26.55	6.57
1 % w/v Brij 35 in water	243.20 ± 17.26	7.09
10 % w/v Propylene glycol in water	390.13 ± 39.38	10.09
10 % w/v Tetraglycol in water	477.76 ± 57.22	11.97

* n = 3, mean ± SD.

Table 5: Fluxes of diclofenac sodium from its 25 mg/ml solution through human placental membrane.

Donor vehicle	Steady state flux* (mg/min cm^2)	% CV
Water	54.34 ± 9.26	17.03
0.05 mg/ml Tween 20 in water	54.66 ± 20.00	36.60
1 % w/v Brij 35 in water	43.79 ± 8.42	19.22
10 % w/v Propylene glycol in water	47.61 ± 27.08	56.90
10 % w/v Tetraglycol in water	32.11 ± 3.36	10.45

* n = 3, mean ± SD.

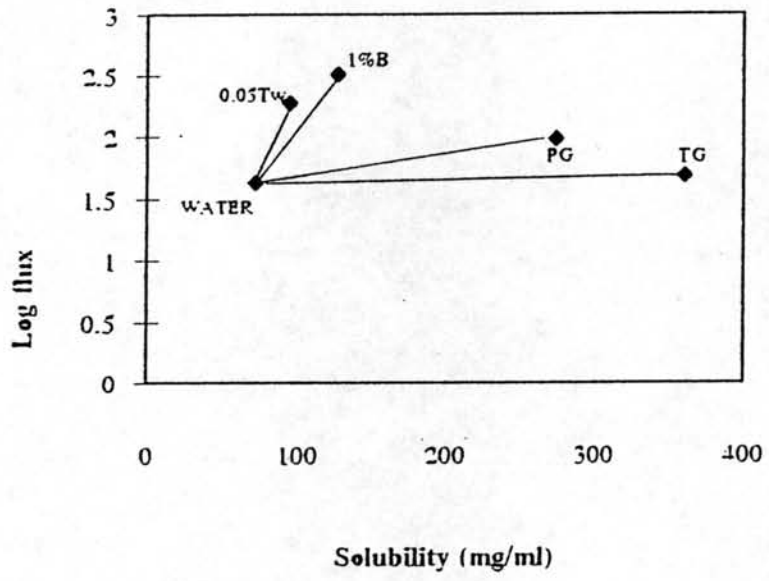

Figure 10: Correlation of steady state fluxes of diclofenac sodium from its 25 mg/ml solution through newborn pig skin and its solubilities.

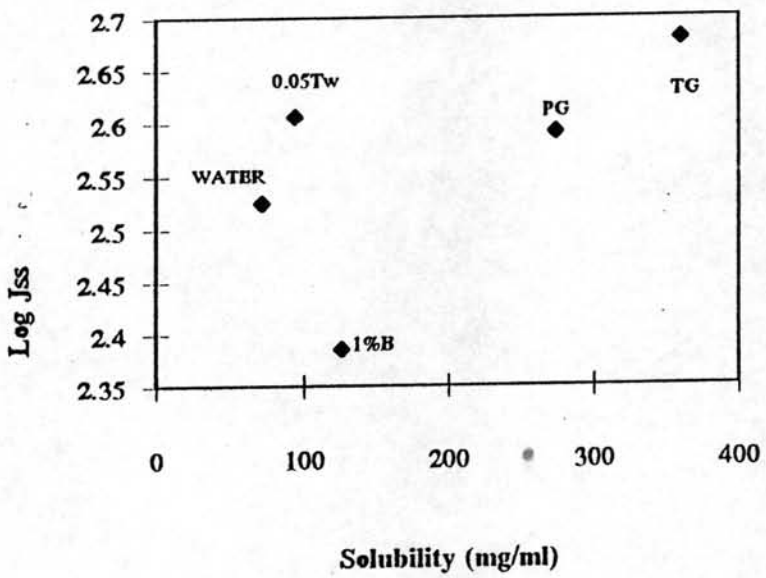

Figure 11: Correlation of steady state fluxes of diclofenac sodium from its 25 mg/ml solution through human amnion and its solubilities.

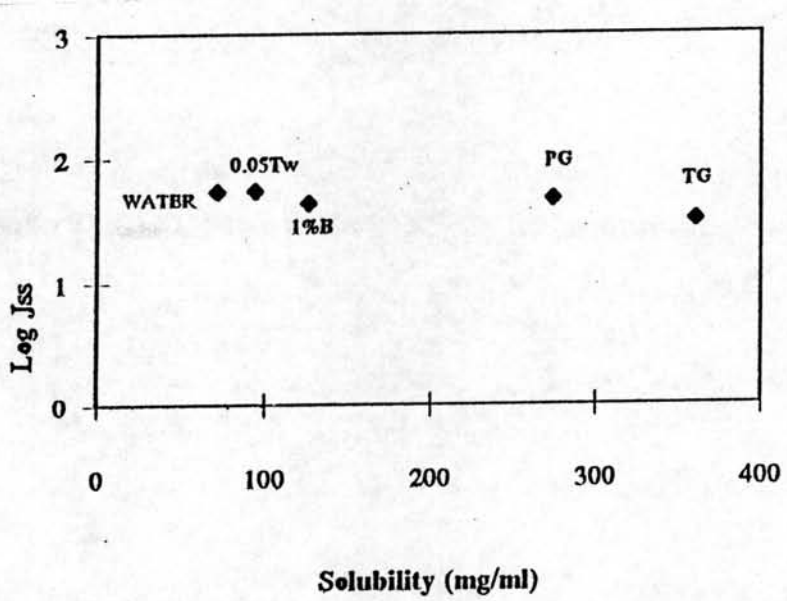

Figure 12: Correlation of steady state fluxes of diclofenac sodium from its 25 mg/ml solution through human placental membrane and its solubilities.

20 in water and 1.0% w/v brij 35 in water) increase the fluxes in greater extents than the glycols (10% propylene glycol in water and 10% w/v tetraglycol in water) because of the more slope values. An explanation is that the glycols increase the solubility of diclofenac sodium more than the surfactants do, so the drug activities in the glycols are less than those in the surfactants and the escaping tendencies are therefore less. The glycols may also act as non-invasive enhancers that alter the skin permeability less than the surfactants. In the glycol group, the flux from 10% w/v tetraglycol is less than the flux from 10% w/v propylene glycol because the drug solubility in 10% w/v tetraglycol is more than that in 10% w/v propylene glycol. From the slopes of the plot, tween 20 should have more enhancement effect than brij 35 since its slope is greater.

The differences in steady state fluxes of 25 mg/ml diclofenac sodium from different vehicles across human amnion (Table 4 and Figure 11) are less than those across pig skin. In the other words, the change in drug activity does not have as much effect on the flux across human amnion as in the case of pig skin. Therefore, the enhancement effect of the vehicles is less in this case, especially, by brij 35. The drug solubility in the aqueous solution of brij 35 is greater than that in water and its flux in the solution of brij 35 is less. Whereas the other vehicles have the opposite results. Therefore, the brij 35 solution has the least effect on human amnion. This is opposite to the case of pig skin. Furthermore, the enhancement effect of tetraglycol solution is greater than that of propylene glycol as its slope value is greater. These suggest some differeness between human amnion and newborn pig skin. The one way ANOVA test (Table 6) indicates that at least one of flux value is different from the others from a statistical standpoint. Table 7 shows the drug flux

Table 6: One way ANOVA for steady state fluxes of diclofenac sodium from its 25 mg/ml solution through human amnion.

Source	df	SS	MS = SS/df	F
Among groups	4	91582.62	22895.66	9.37
Within group	10	24417.50	2441.75	
Total	14	116000.10		

F table (4,10) = 3.48

Level of significant = 0.05

df = degree of freedom

SS = sum of square

MS = mean square

F = variance ratio

Table 7: Duncan' s new multiple range test.

Least Significant Ranges

LSR , P = 2	89.86
LSR , P = 3	94.14
LSR , P = 4	96.14
LSR , P = 5	97.85

Means

#1	=	243.20	1% w/v Brij 35 in water
#2	=	334.33	Water
#3	=	390.13	10% w/v Propylene glycol in water
#4	=	404.03	0.05 mg/ml Tween 20 in water
#5	=	477.76	10% w/v Tetraglycol in water

1 2 3 4 5

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_____

Significant = Water V.S. 1% w/v Brij 35 and 10% w/v Tetraglycol

Non significant = Water V.S. 0.05 mg/ml Tween 20 and 10% w/v Propylene glycol

differences between water and 1% w/v Brij 35 and between water and 10 % w/v tetraglycol.

From a statistical standpoint, the fluxes of drug in all vehicles through human placental membrane are not different since the F statistic (1.29) is less than F (4,10 $p < 0.05$) = 3.48 (Table 8). Therefore, the enhancers have the least effect on human placental membrane among the three membranes studied. Since the surfactants and glycols are capable of extracting a lipid which is a major barrier of diffusion through pig skin, the lipid content in human placental membrane should be much less than that in pig skin.

3.2 Saturated Solutions of Diclofenac Sodium as a Donor Phase.

From Fick's First law, an alternative way to consider the relationship defined by this law is to express it in terms of thermodynamic activity (Higuchi, 1960):

$$\frac{dM}{dt} = J = \frac{aD}{\Upsilon h} \quad (11)$$

where a is the thermodynamic activity of drug in its vehicle and Υ is the effective activity coefficient of drug in the skin barrier phase. If this relationship is generalized with respect to time, the drug flux should be directly proportional to the thermodynamic activity in the vehicle. Since the thermodynamic activity of drug is the same in all saturated solutions, the same flux should be achieved from all saturated solutions under the condition that the vehicles do not damage the skin or otherwise

Table 8: One way ANOVA for steady state fluxes of diclofenac sodium from its 25 mg/ml solution through human placental membrane.

Source	df	SS	MS=SS/df	F
Among groups	4	1345.64	336.41	1.29
Within groups	10	2601.22	260.12	
Total	14	3946.46		

F table (4,10) = 3.48

Level of significant = 0.05

df = degree of freedom

SS = sum of square

MS = mean square

F = variance ratio

alter the skin permeability during an experiment (Dugard, 1986).

3.2.1 Diffusion through Newborn Pig Skin.

The normalized fluxes of diclofenac sodium from its saturated solutions through newborn pig skin are shown in Table 9. The general rank order of the donor vehicles that lead to the steady state fluxes from the lowest to the highest value is: ethanol < 0.4 % w/v brij 35 in water < 1 % w/v orange oil in ethanol < water < isopropanol < 1% brij 35 in water < 0.01 mg/ml tween 20 in water < 0.05 mg/ml tween 20 in water < 10 % w/v tetraglycol in water < 10% w/v propylene glycol in water. The fluxes obtained are not the same inspite of using saturated solutions which is not correlated to the assumption mentioned previously.

From a theoretical point of view, a plot of flux and solubility of drug should be a straight line parallel to the solubility axis. However, the fluxes of drug change in different vehicles (Figure 13). This obviously indicated that the vehicles must interact with the pig skin and alter the diffusional barrier of the membrane.

In the case of aqueous solutions, the fluxes increase with increasing the solubilities of drug except for the case of drug in 0.4 % w/v brij 35. Since the normalized fluxes and saturated solutions are used, the membrane thickness and donor concentration should not be considered here. The only parameters that are responsible for the differences in fluxes are the membrane/donor vehicle partition coefficient (Table 2) and the diffusion coefficient of drug in the membrane. The diffusion coefficients of drug were calculated using Eq. 5 and are presented in Table

Table 9: Steady state fluxes of diclofenac sodium from its saturated solutions through newborn pig skin.

Donor vehicle	Steady state flux * (mcg/min cm^2)	% CV
Water	163.96 ± 162.50	99.16
0.01 mg/ml Tween 20 in water	332.50 ± 117.10	35.40
0.05 mg/ml Tween 20 in water	699.90 ± 374.50	53.50
0.4 % w/v Brij 35 in water	98.16 ± 16.32	16.62
1 % w/v Brij 35 in water	259.33 ± 120.30	46.38
10 % w/v Propylene glycol in water	1867.73 ± 649.30	34.76
10 % w/v Tetraglycol in water	1036.23 ± 801.10	77.31
Ethanol	60.22± 26.39	43.83
Isopropanol	226.90 ± 41.42	18.25
1 % w/v Orange oil in ethanol	116.73 ± 23.78	20.37

* n = 3, mean ± SD.

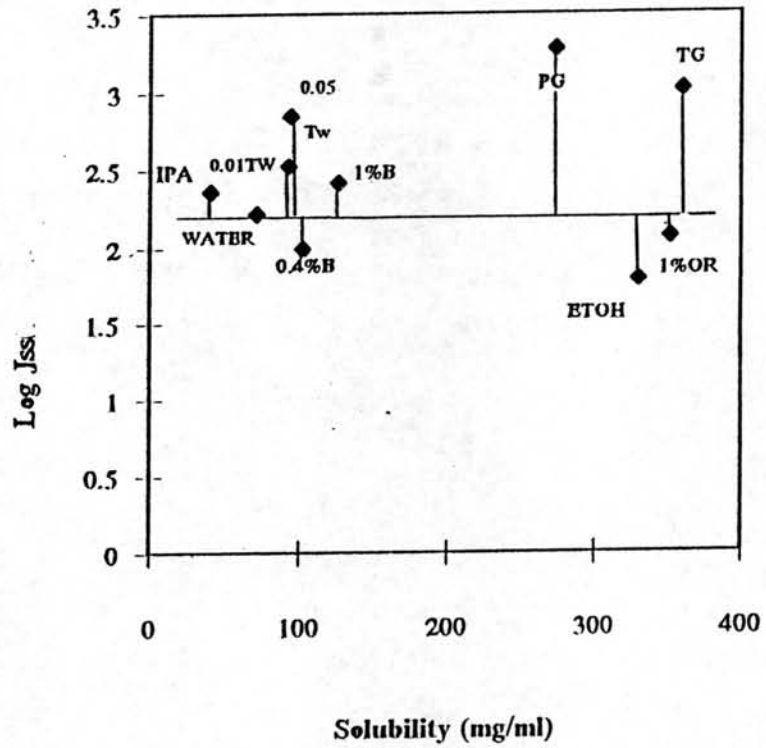

Figure 13: Correlation of steady state fluxes of diclofenac sodium from its saturated solutions through newborn pig skin and its solubilities.

Table 10: Diffusion coefficients of diclofenac sodium in the three membranes.

Donor vehicle	Diffusion coefficient(cm^2/min)		
	Pig skin	Human amnion	Human placental membrane
Water	1.020	1.599×10^{-3}	5.541×10^{-3}
0.05 mg/ml Tween 20 in water	0.318	23.900×10^{-3}	7.689×10^{-3}
1.0 % w/v Brij 35 in water	0.056	0.799×10^{-3}	6.997×10^{-3}
10 % w/v Propylene glycol in water	0.056	1.957×10^{-3}	2.252×10^{-3}
10 % w/v Tetraglycol in water	0.038	0.668×10^{-3}	2.877×10^{-3}

* n = 3, mean $\pm$ SD.

10. The increase in fluxes results from the increases in both partition coefficient and / or the diffusion coefficient.

Tween 20 has the critical micelle concentration (CMC) of 0.04 mg/ml (Zatz and Pramod, 1987). Both the concentration of tween 20 below its CMC (0.01 mg/ml) and above its CMC (0.05 mg/ml) causes the increase in fluxes. This indicates that the monomer of tween 20 also helps the movement of drug through the skin. Since the flux of drug from 0.05 mg/ml tween 20 solution is greater than that from 0.01 mg/ml tween 20 solution, the micelle of tween 20 must increase the diffusion rate of drug. Brij 35 has the critical micelle concentration of 0.5 % w/v. When its concentration is below its CMC (0.4 % w/v), the flux of drug is less than the flux of drug in water. In contrary, the concentration of Brij 35 that is above its CMC (1 % w/v) increases the flux. Therefore, the monomer of Brij 35 must retard the diffusion rate of drug. Brij 35 (C12E23) might have too long epoxy chain (with hydrophilic head groups) to incorporate well into the lipophilic stratum corneum. When the micelle is formed, its hydrophilic groups are shielded and therefore, the micelle carrying the drug can move across the skin more easily.

When the flux increments by the micelles of tween 20 and Brij 35 are compared, the micelle of tween 20 would aid the diffusion of drug more than that of Brij 35 would since the flux increment by 0.05 mg/ml tween 20 is greater than by 1 % Brij 35. There is an evidence of the higher value of diffusion coefficient of drug when tween 20 is included in the donor solution.

The enhancement effect of the glycols mostly comes from its increment of partition coefficients of drug. Since 10 % w/v propylene glycol yields the highest flux, its role as an enhancer should be the greatest among the

enhancers studied. Its mechanism is by helping the drug partitioning into the skin in the greatest extent. The flux of drug from 10 % w/v tetraglycol is less than that from 10 % w/v propylene gylcol because of its lower partition coefficient value.

The glycols enhance the diffusion rate of drug across pig skin more than the surfactants do. And it seems that the partition coefficient increment is responsible for the diffusion rate enhancement in the case of pig skin.

Ethanol and 1 % w/v orange oil in ethanol lead to lower fluxes than the flux from water. This may be resulted from the effect of concentration of ethanol. Berner et. al.(1989) studied skin penetration of nitroglycerin in ethanol. He found that ethanolic volume fraction of 0.7 or below improved the drug flux across human skin but a higher alcoholic concentration inhibited the penetration. This is agreed with the result in this study because ethanol used in this study was absolute ethanol (99.7 %) and the orange oil solution also contained ethanol up to 99 % by volume. The dehydration of stratum coneum by a high concentration of ethanol and other lower alcohols results in a reduction of drug penetration across the skin. The flux retardation by 1% w/v orange oil in ethanol is less than that by ethanol which indicated that 1% w/v orange oil can act as an enhancer.

The flux of drug in isopropyl alcohol is more than that in water and ethanol. Scheuplein (1965) studied the absorption of a homologous series of alcohols (methanol to octanol) through the mouse skin. He found that the addition of each $-CH_2-$ group resulted in a large increase in permeability by increasing the partitioning of alcohols from the vehicle into the barrier. Therefore, isopropyl alcohol

should lead to a higher partition coefficient of drug than water and ethanol do and thus increase the steady state flux.

3.2.2 Diffusion through Human Amnion.

The normalized fluxes of diclofenac sodium from its saturated solutions through human amnion are presented in Table 11. The general rank order of the donor vehicles that yield the steady state fluxes from the lowest to the highest value is: isopropanol < ethanol < 1 % w/v orange oil in ethanol < 0.4 % w/v brij 35 in water < 1.0 % w/v brij 35 in water < water < 0.01 mg/ml tween 20 in water < 0.05 mg/ml tween 20 in water < 10 % w/v tetraglycol in water < 10 % w/v propylene glycol in water.

As it was stated previously that the slope of log Jss versus drug solubility plot (Figure 14) should be zero if the vehicles did not have any effects on the membrane. In this case the effect of the aqueous vehicles is less than the case of pig skin because of the less scattering of the fluxes around the zero-slope line. Therefore, the amnion is altered by these vehicles less than the pig skin.

The enhancement effects of tween 20, propylene glycol and tetraglycol solutions are similar to the case of pig skin but with a lesser extent. Since all vehicles lead to lower partition coefficients of drug than water (Table 2), all enhancement effects should be from the increment of the diffusion coefficients. The glycols enhance the diffusion rate of drug more than tween 20 because of having more partition coefficient value. Both 0.4 % w/v and 1 % w/v brij 35 retard the steady state flux since their effects on the diffusion coefficient are low.

Table 11: Steady state fluxes of diclofenac sodium from its saturated solutions through human amnion.

Donor vehicle	Steady state flux * (mg/min cm^2)	%CV
Water	229.83 ± 55.24	24.03
0.01 mg/ml Tween 10 in water	245.23 ± 75.74	30.88
0.05 mg/ml Tween 20 in water	321.30 ± 51.18	15.93
0.4 % w/v Brij 35 in water	107.56 ± 26.34	24.53
1 % w/v Brij 35 in water	141.63 ± 56.34	39.79
10 % w/v Propylene glycol in water	537.06 ± 146.30	27.25
10 % w/v Tetraglycol in water	357.26 ± 135.00	37.79
Ethanol	41.65 ± 1.08	2.60
Isopropanol	3.01 ± 0.56	18.64
1 % w/v Orange oil in ethanol	47.14 ± 20.23	42.90

* n = 3, mean ± SD.

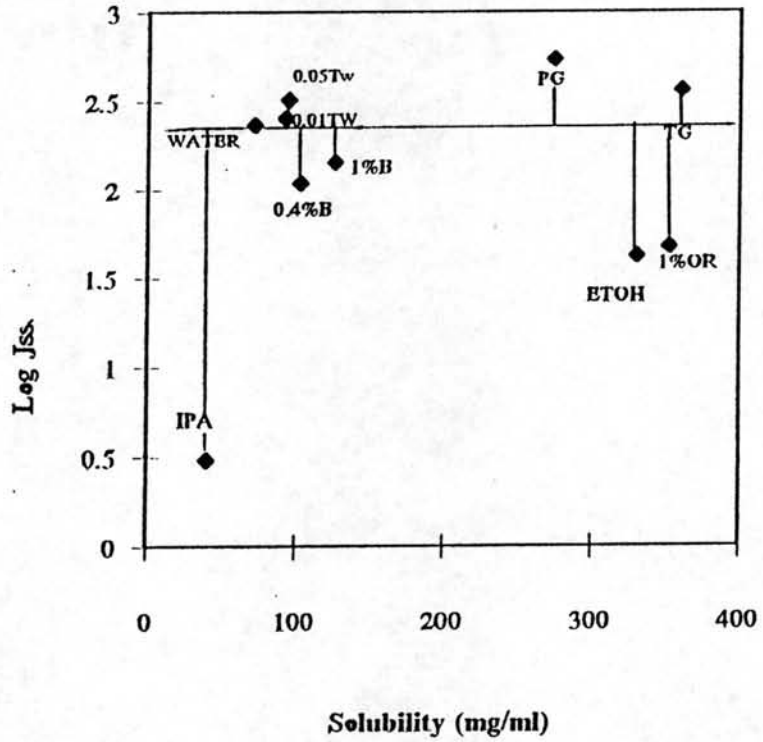

Figure 14: Correlation of steady state fluxes of diclofenac sodium from its saturated solutions through human amnion and its solubilities.

The amnion may behave more like a pore membrane since it composes of epithelial cells with microvilli. An evidence is the less partition coefficients of drug when it is in all aqueous vehicles other than water. At the beginning of an experiment, the pores were filled with the aqueous receiving solution. After the donor solution had been applied, the movement of the vehicle into the pore lowered the solubility of drug comparing with the drug solubility in the vehicle since the drug is less soluble in the aqueous solution of more water content (the least solubility of drug in the aqueous solutions is in water).

All alcoholic solutions retard the diffusion rates especially isopropanol. They should dehydrate the membrane and decrease the partition coefficient of drug. They may also shrink the pores of membrane and thus reduce the amount of drug permeating.

3.3.3 Diffusion through Human Placental Membrane.

The normalized fluxes of diclofenac sodium from its saturated solutions through human placental membrane are presented in table 12. The general rank order of the donor vehicles that yield the steady state fluxes from the lowest to the highest value is: isopropanol < 1 % w/v orange oil in ethanol < 0.4 % w/v brij 35 in water < ethanol < water < 0.01 mg/ml tween 20 in water < 0.05 mg/ml tween 20 in water < 1 % w/v brij 35 in water < 10 % w/v propylene glycol in water < 10 % w/v tetraglycol in water.

The plot of log Jss versus the drug solubility (Figure 15) shows similar deviations of the aqueous donor vehicles from the zero-slope line to the

Table 12: Steady state fluxes of diclofenac sodium from its saturated solutions through human placental membrane.

Donor vehicle	Steady state flux * ($mg/min\ cm^2$)	% CV
Water	40.34 ± 5.09	12.62
0.01 mg/ml Tween 20 in water	55.75 ± 18.99	34.07
0.05 mg/ml Tween 20 in water	68.08 ± 23.92	35.13
0.4 % w/v Brij 35 in water	36.47 ± 15.17	41.61
1 % w/v Brij 35 in water	77.85 ± 4.04	5.19
10 % w/v Propylene glycol in water	95.51 ± 27.42	28.71
10 % w/v Tetraglycol in water	132.50 ± 37.72	28.46
Ethanol	39.29 ± 4.79	12.18
Isopropanol	3.94 ± 1.78	45.30
1 % w/v Orange oil in ethanol	34.50 ± 9.03	26.18

* n = 3, mean ± SD.

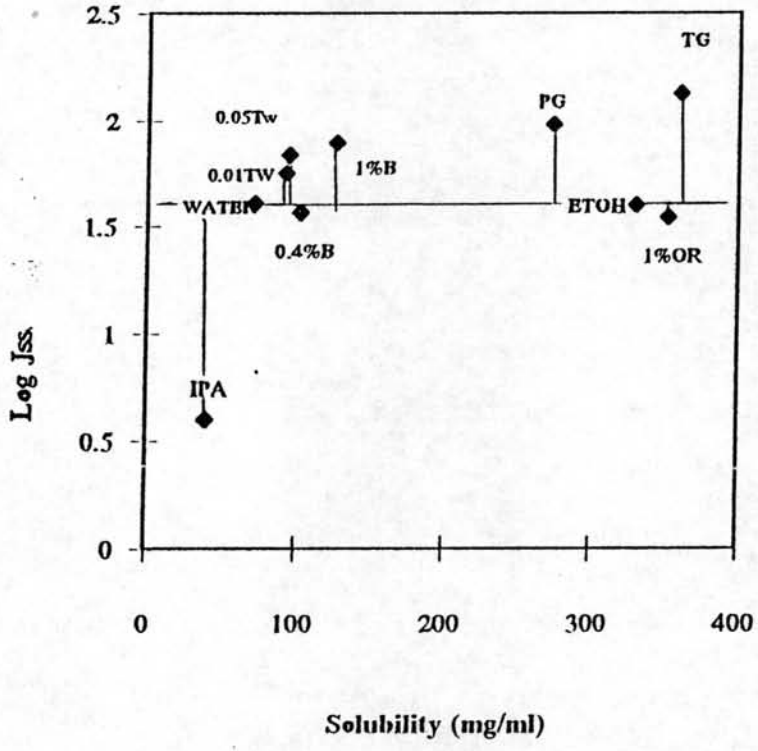

Figure 15: Correlation of steady state fluxes of diclofenac sodium from its saturated solutions through human placental membrane and its solubilities.

case of pig skin except that tetraglycol mixture yields the higher flux than propylene glycol mixture and 1% w/v brij 35 yields the higher flux than tween 20. This is because tetraglycol leads to a higher diffusion coefficient than propylene glycol. Both propylene glycol and tetraglycol lead to lower diffusion coefficients than water (Table 10). These should be the reason why propylene glycol does not give the highest flux.

The higher enhancement effect by the glycols than the surfactants is also from the higher increase in partition coefficient of drug. The enhancement effect by the surfactants is also by the increment of both the diffusion coefficients and the partition coefficients. The effects of tween 20 is the same as those in the case of pig skin.

The retardation effect by the alcoholic vehicles is similar to that in the case of amnion except that 1 % orange oil retard the diffusion rate more than ethanol alone.

Human amnion and placental membrane contain less lipid content than newborn pig skin and the mode of action of enhancer mostly involves in the extraction of lipid or fluidization of lipid structure and thus the enhancing effect on human amnion and placental membrane is less than that on newborn pig skin.