CHAPTER IV

DISCUSSION AND CONCLUSIONS

In Vitro Evaluation of NFP Transdermal Patch

Physical properties

Owing to the physical properties data in Table 2, the control formulation (F1) containing 1% w/w NFP, 50% w/w Pluronic F-127 gel matrices, 10% w/w glycerin, and 6% w/w Aerosil A-200 exhibited a good physical appearance. Pluronic F-127 gel was used as hydrophilic gel matrices that exhibited sustained release of NFP over 24 hours with a constant rate (Tattawasart, 1992). Glycerin was used as a polymer plasticizer to reduce stiffness of polymer backbone and increase the flexibility of polymer chain. Accordingly, this would increase drug diffusion rate from devices. Aerosil was used as viscosity modifier for increasing consistensy of gel matrices.

The addition of such surfactants using as permeation enhancers affected to physical appearance of NFP gel matrices.

Difficulty in preparing

According to the characteristic of each surfactant, it was noticed that the characteristic of surfactants affected to the difficulty in preparing of NFP gel matrices. Since Cremophor A 25, cetyl and stearyl alcohol were unctuous and hydrophobic substances, they were slowly dissolved in a hydrophilic gel base. Eventhough, they were size reduced by

passing through sieve no. 80 before use, it took longer times to disperse these surfactants completely into Pluronic F-127 gel.

Clarity

Each ingredient in all formulations could dissolved to form homogeneous gel matrices. The reasons due to the both hydrophilic and hydrophobic properties of Pluronic F-127 gel base include excellent compatibility with other ingredients (BASF, 1987) and form non-ionic micelles in gels which are assumed to consist of large population of micelles (Chen-Chow, 1981; Tomida, Kuwada, and Kiryu, 1988). The characteristics and shape of micelles are dependent on temperature. At low temperature Pluronic F-127 can form unimiceile, single molecule of Pluronic F-127 per micelle, and when the temperature up to 10 °C and over, Pluronic F-127 will form micelles of polymolecules as six molecules per micelle (Schmolka, 1991). Due to its properties in micelle formation, Pluronic F-127 can improve solubility of purely water soluble drug by micellar interaction (Tomida, Kuwada, and Kiryu, 1988). Thus, all ingredients might increased their solubility by accommodated into the interior of the micelles of Pluronic F-127 aqueous gel matrices.

Air bubbles

Small air bubbles were prominent in some formulations. These may be due to the longer times used in preparing, then surfactants could reduced interfacial tension between gel and gas. The unbalanced force allow gas trapped into gel and forms air bubbles. Generally, a reversible gel (such as Pluronic F-127) could be release air bubbles which accidentally incorporated during processing (BASF, 1987).

Residue on application

The residue of gel matrices when applied on the surface of skin were related to the consistency of each preparation. The consistency was not dependent on surfactant type. However, the concentration of some surfactants affected the consistency of preparation whereas some did not. Such as sodium lauryl sulfate, it was found that increasing concentration of SLS decreased consistency of the preparation. This result was found in formulations containing Cremophor RH40 since it was thin paste and decreased viscosity of gel base during processing.

The ten designed formulations could exhibited overall present physical appearance and were selectd to further *in vitro* study.

***In vitro* evaluation of NFP TDDs formulations**

The higher correlation coefficient in the relationship between cumulative NFP permeation against time of all formulations were observed in this study. The first phase, showing the lower NFP permeation rate from each formulation could attributed to the complicated permeation process which involved the process of drug releasing and the effect of skin uptake on the permeation of drug through pig's skin (Chien, 1987, Wan, Heng and Wong, 1990, Urban et al., 1991). The process of drug releasing from a matrix was a complex one. This involved water penetration into the drug matrix, hydration and gelation of the polymer, diffusion of the dissolved NFP in the resultant gel and erosion of the gel layer. After the pig's skin was saturated with the drug molecules, the rate of NFP delivery did not change by the effect of skin uptake. This was contribution to the higher permeation rate of drug delivery of the second phase (10-24/28 hours) than the first phase.

In NFP transdermal patch containing anionic surfactant (sodium lauryl sulfate), it was found that the permeation amount of NFP were higher than control formulation (F1). It may be due to the effect of anionic surfactant which could penetrate and interact strongly with skin (Bettley, 1965; Gibson and Teall, 1983). Exposure of the skin to SLS changed the lipid composition of the stratum corneum, whereby it diffusivity was changed. This was further supported by the continuous increase in permeation rate during the exposure, i.e., the absence of steady state.

In this study, the permeation amount of NFP was not linearly related to SLS concentration. Amount from formulation containing 0.5% SLS was the highest permeation in this group. With 0.5 % SLS, this level might be closely to the critical micelle concentration (CMC). Thereby the formulation containing 0.5 % SLS was observed to exhibit higher NFP amount than the formulations containing 1.0 and 2.0% SLS. These results were in agreement with the study of Loden (1990).It was found that, above the CMC the added surfactant was supposed to exit in the solution as micelles. These micelles did not penetrate the skin, due to the combination of size and negative charge. Thus, the monomer activity of SLS might not be constant above critical micelle concentration (CMC) but might increased when the concentration of micelles increase . Above the CMC, the SLS activity seemed to be proportional to the increase in concentration. Thus, a practical outcome might be that skin damage from SLS was not linearly related to the concentration. The rate of penetration also increased with respect to time, indicating increasing damage to the skin over the duration of contact. Chowhan and Pritchard (1978) found that, above of CMC, proportionaltely less surfactant was available for interaction and SLS increased the *in vitro* flux of naproxen appreciably.

The enhancing mechanism of cationic surfactant was generally observed to form an ion pair with the drug. The ion pair, which has an intrinsically higher solubility in the skin than does the parent anion, diffuses down its own concentration gradient to the inner layers of the stratum corneum. In this region the pH is 7.4, and the amine deprotonates, liberating the anion. Once it has deprotonated, the amine is free to travel back to the skin surface(Barker and Hadgraft, 1981).

However, Tan et al (1993) found that quarternary ammonium compounds could enhance the skin permeation of indomethacin to vary magnitudes and through different mechanisms. Some involved increased drug solubilization in the aqueous vehicle while others demonstrated permeability increase as a function of partitioning..

In NFP transdermal patch containing cationic surfactant, the different permeation amount of F5, F6 containing 1 and 2 % cetyltrimethylammonium bromide, respectively, might attribute to the effect of surfactant on pig's skin. The high concentration of cetyltrimethylammonium bromide might affect the NFP permeated amount by altering the barrier function of skin. By the means, it might bind readily to epidermal protein whereas the lower concentration exhibited the less effect.

The similar trend and the fairly close in magnitude of F7 and F8 with 1 and 2% cetylpyridinium chloride, respectively, in comparison with the control formulation (F1) contributed that this surfactant had no membrane permeability-enhancing effect.

For NFP permeation amount from formulations containing nonionic surfactant showing lower value than control formulation, the possible reason could be explained by a consideration of their surface properties. By the

meaning, the monomers of this group might interact with skin, then alter the barrier properties. The micelles, however, have a marked solubilizing capacity which for interacting molecules could lead to a significant decrease in permeant thermodynamic activity in the vehicle. Black and Howes (1989) discovered that the mode of action of non-ionic surfactants appeared to be linked to their ability to increase membrane fluidity and their capacity to solubilize and extract membrane components.

***In Vivo* Evaluation Study**

Method of analysis

In this study, The chromatographic conditions were modified to suit rabbit plasma. Mobile phase containing 0.05M acetate buffer gave the better NFP shape peak than previously reported of 0.01 M acetate buffer.

By modifying the ionic strength of mobile phase to get the better resolved chromatogram, the method has already been proven to sufficiently be simple, sensitive, and specific enough for determining NFP in rabbit plasma.

Single-oral dose application

As shown in Figure 24, the time to reach maximum plasma drug concentration (t_{max}) following oral drug administration to rabbit appeared to vary widely from 0.5 hr to12 hr. A wide variation of the t_{max} values in this study would probably be due to various reasons. Except the intersubject variations that are usually occurred, one of the main reason for the difference in t_{max} values should be because of the experimental error in feeding NFP to rabbits. Actually, drug have to be oral administered to animal via feeding tube that direct the drug to the GI tract. In this experiment, drug was forced

into rabbit's mouth with a 10 ml of drinking water. There is no garrantee whether the drug was swallowed into the stomach, that's why the t_{max} of Rabbit No. 2 and 5 were at 12 hr. However, the t_{max} value of Rabbit No.1 and 3 were similar to those reported by Kohri (2 hr) and weren't also difference from human studies (0.5-2 hr). Therefore, it might be said that oral NFP administration to rabbit gave similar t_{max} value comparing to other studies if the drug was directly fed into the GI tract. It was also suggested that experiment on oral drug administration to animal should not be performed unless the tools for feeding drug use available in the laboratory.

The NFP concentration at t_{max} as shown in Table 6 also correspond to the aforementioned reason on feeding drug. The C_{max} value for Rabbit No.2 and 5 were just very small compared to the other three subjects. This confirms the possibility of not having drug directly to GI tract.

NFP transdermal patch application

The selected NFP transdermal patch experimented on rabbits showed the same t_{max} for all rabbits studied. This suggests the less intersubject variation for transdermal dosage form than oral administration. The plasma NFP concentration-time profiles of two out of five rabbits (No.1 and 4) showed sustain released pattern of drug from dosage form up to 24 hrs. This implies that the sustain release formulation prepared in this study could possibly be accepted. However, the result have to be confirmed with more animal studies. The variation of C_{max} values, not only due to intersubject variation as usually be, but also imply the release pattern of drug from studied dosage form. Comparing to oral dosage form with same unit dose, C_{max} values for transdermal patch are lower than from oral dosage form.

The significant higher AUC $|_{0}^{24}$ of oral NFP administration than that of transdermal patch administration inform the possibly approvement of transdermal patch formulation to get the better released drug in systemic circulation. Many reasons of small release of NFP from transdermal patch would possibly be due to the limit absorption of drug through rabbit skin, the condition of skin or any other variations. All of these are waiting to be proven in the future.

***In vitro - In vivo* relationship**

According to Table 9, the release pattern of NFP from transdermal patch into diffusion cell was higher significant difference than that released into rabbit circulation. It might be expected to the composition of rabbit skin, especially stratum corneum, played the role as rate limiting barrier of drug absorption, whereas the more amount of NFP permeation were observed from *in vitro* study. It is suggested that the more data should be collected with more number of sample experiments to confirm the final conclusion.

Conclusions

In summary, all transdermal patches exhibited yellowish transparent gel with good consistency, disappearance of air bubbles prominent, and easy in preparing.

For *in vitro* skin permeation study, both the types and concentration of surfactant apparently affected the NFP permeation amount. Obviously, 2 % cetyltrimethylammonium bromide, a cationic surfactant, showed the enhancing effect providing the highest NFP permeation amount whereas 1% cetyltrimethylammonium bromide evidently exhibited the lowest value. Other surfactants showed the difference enhancing effect due to themselves

properties. The skin permeation amount achieved from the formulations contained various concentration of cetylpyridinium chloride were similar to control formulation. For anionic surfactant, preparation composed of 0.5 % sodium lauryl sulfate exhibited greater skin permeation amount than other formulations in this group. Increasing concentration of Cremophor A25 was decreased NFP permeation amount. The first phase, showing the lower NFP permeation rate from each formulation could attributed to the complicated permeation process which involved the process of drug releasing and the effect of skin uptake on the permeation of drug through pig's skin.

The experiment of using NFP transdermal patch in rabbit is clearly shown that NFP could be released from transdermal patch into systemic circulation. The NFP plasma concentration time profiles of two from five rabbits showed sustained-released pattern up to 24 hrs. However, the formulations showed long lag time before drug concentration can be detected in the circulation. In addition, the release amount of NFP from transdermal patch in rabbit circulation was less than that from oral in the same dose. It is therefore suggested that the formulation would have to be modified in the further study.