Chapter V

Conclusion

The results from this study can be summarized as follows:

1. The four diclofanac diethylammonium gel products (A, B, C and D) contained equivalent amount of the active ingredient, with the percent labeled amount falling in the generally acceptable range of 95.0-105.0 % for all products.

2. The *in vitro* diffusion experiments employing the modified Franz diffusion cell apparatus indicated that the synthetic porous membranes such as cellulose acetate and Durapore® membranes did not have significant barrier properties against drug diffusion. The cellulose acetate membrane is a hydrophilic membrane which can be easily wetted in an aqueous medium of the receiver compartment. As a result, it is suitable for use in the *in vitro* diffusion experiments to investigate the release characteristics of diclofenac diethylammonium gel products. Durapore® membrane, on the other hand, is a lipophilic porous membrane made from polytetrafluoroethylene (PTFE). It is more difficult to wet in an aqueous medium and therefore should be prewetted in an appropriate solvent such as alcohol prior to the diffusion experiments so that valid interpretation of the release profiles can be made.

3. The amount of diclofenac diethylammonium in the receiver compartment of the diffusion apparatus was analyzed by a specific HPLC technique employing phenylbutazone as an internal standard. Release profiles for each gel product were obtained by plotting the cumulative amount released as a function of the square root of time in order to determine the release rate according to the Higuchi equation. Two *in vitro* parameters were calculated for each product from these plots, namely the cumulative amount released at 6 hr and the release rate.

4. Comparison of these *in vitro* parameters using ANOVA revealed that there was a significant difference ($p < 0.05$) among the four products tested with respect to the cumulative amount released at 6 hr through cellulose acetate membrane. Duncan's test was further applied to rank these four products. It was found that products A and C significantly released diclofenac to a greater extent than the products B and D. However, there was no significant difference between A and C or between B and D. As a result, the four products can be roughly classified into two groups, i.e. one group with greater amount of drug release (A and C) and another group with lesser amount of drug release (B and D).

5. On the other hand, ANOVA did not show any signficant difference in the release rate among the four products through the cellulose acetate membrane ($p > 0.05$). However, the values of the release rate for products A and C still greater than products B and D.

6. The diffusion experiments with the Durapore® membrane gave drug release profiles which were less than the cellulose acetate membrane for all the

four products. ANOVA showed significant differences among the four products in both the release rate and the cumulative amount of drug release through this membrane ($p < 0.05$). However, similar rankings into two distinct groups of A,C and B,D could not be made.

The observed discrepancies could be due to the lipophilic nature of the Durapore® membrane. Durapore® was not prewetted with alcohol in all the diffusion experiments and thus the aqueous receiver fluid may not be in full contact with the membrane surface. In addition, the composition of the gel base for the commercial products A, B and C was not known, exept the experimental product D (see Appendix I for formulation). These commercial preparations contained varying amount of alcohol and thus the extent to which the gel product wetted the membrane surface on the donor side of the diffusion cell was also not known. Trial experiment has shown that when Durapore® was prewetted with alcohol, the rate and extent of drug release was restored to the level comparable to that of the hydrophilic cellulose acetate membrane.

7. Shed snake skin specimens from *Elaphe obsoleta* were selected as a representative animal membrane for the *in vitro* permeation experiments on the basis of their resemblance to the human stratum corneum. The skin consists of pure stratum corneum which can be readily cut and fitted to the diffusion apparatus. The membrane was presoaked overnight in the receiver fluid to allow for complete hydration and swelling before use. Permeation profiles were obtained by plotting the cumulative amount of drug found in the receiver compartment as a function of time. The cumulative amount permeated through this skin membrane at any time point was much lower than that observed with the cellulose acetate and Durapore® membranes. This indicated that, in

contrast to the porous membranes, the shed snake skin possessed a significant barrier property which substantially retarded the penetration of diclofenac.

ANOVA results also showed that there were significant differences in both the amount of diclofenac penetrated and the steady state permeation flux among the four products ($p < 0.05$). However, the rankings after Duncan's test were much different from the cellulose acetate data. This was not unexpected since the shed snake skin acted as an important barrier against drug penetration as opposed to the porous cellulose acetate membrane. Two processes were occurring simultaneously during the percutaneous absorption of diclofenac through the shed snake skin, i.e. the release of drug from the vehicle as well as its penetration through the membrane barrier. The latter process could be the rate limiting step which may have altered the ranking sequence of the four products when compared to the cellulose acetate membrane.

8. A technique to evaluate the *in vivo* percutaneous absorption of topical diclofenac diethylammonium gel products has been developed in this study based on analysis of the drug in the stratum corneum at various times after drug application. Briefly, an excess amount of the gel product was topically applied to the designated area of the body, i.e. the left or right forearm. The area of the forearm was further divided into several row of squares (1 x1 cm dimension). Each square was called an application spot on which the small amount of gel was applied. After drug application, each spot was then occluded with Tegaderm tape for certain period of time. Following the occlusion period, the excess product was removed from each spot to terminate the drug application. After that, the skin stripping of each spot took place using a series of adhesive tape strips (Transpore®) to remove the outer layers of the skin (the stratum corneum). The tape strips were then pooled

together for each spot, immersed in a mobile phase and subjected to analysis for diclofenac content by HPLC.

9. Preliminary stripping experiments have shown that ten consecutive strippings per spot were adequate to remove the stratum corneum from this area. The first two strips were discarded to eliminate any unpenetrated gel product still remaining on the surface of the stratum corneum. The subsequent eight strips were then combined for analysis of diclofenac which has penetrated the stratum corneum and remained in this layer.

Preliminary experiments also showed that the occlusion period of three hours appeared to give optimum amount of diclofenac in the stratum corneum. As a result, each gel product was topically applied under occlusion for 3 hr before temination of drug treatment in all subsequent experiments.

Pilot experiments also demonstrated that, under similar occlusion period, there were no significant differences in the amount of diclofenac found in the stratum corneum between the left and right forearm. Furthermore, there were no significant differences in the amount of drug found in each spot within the same row of the same forearm. This indicated that there was no influence from the left- or right-hand side of the forearm on the amount of drug to be detected. There was also no influence from the different spots within the same row of drug application.

10. After the preliminary study to establish the optimum test conditions, the *in vivo* skin stripping technique was applied in the evaluation of the four diclofenac gel products for their *in vivo* percutaneous absorption and

topical bioavailability. These were the same products which have been previously tested *in vitro* for their release/permeation characteristics.

The *in vivo* study was a crossover, randomized block design. Eight subjects participated in the study. Each of them received all the four products on different occasions separated by about one week washout period. The treatment sequence was randomized and the right or left forearm was also randomly chosen for drug application. Each product was applied to each subject's forearm under occlusion for 3 hr. The area of the forearem was divided into four parallel rows, each row consisting of two application spots, making a total of eight spots. After 3 hr, the excess drug was removed from all the spots to terminate the drug application. Then, one row of spots was randomly chosen and immediately stripped to determine the amount of diclofenac initially found in the stratum corneum following termination of drug treatment (time 0 hr). After initial stripping, each of the remaining rows was then randomly selected for stripping at time 1, 3 and 6 hr to determine the amount of diclofenac remaining in the stratum corneum at these time points. Loss of diclofenac from the stratum corneum during this period should indicate percutaneous absorption of the drug to deeper skin layers and underlying tissues.

11. Statistical analysis of the amount of diclofenac found in the stratum corneum (ANOVA and Duncan's test) indicated that there were significant differences among the four products at all time points, particularly at 0, 1 and 3 hr ($p < 0.05$). The four products can be roughly classified into two groups in a manner similar to the *in vitro* release data from the cellulose acetate membrane, i.e. a group with higher amount of drug found in the stratum corneum (A and C) and a group with lesser amount of drug found in the skin (B

and D). Comparison of the ranking results between the *in vitro* release and the *in vivo* skin stripping gave a rough indication that there might be some correlation between the release of diclofenac from the vehicle and the overall percutaneous absorption.

12. The percent drug percutaneous absorbed from the stratum corneum was also calculated for each product at each time point (1, 3 and 6 hr). ANOVA was applied to these data and showed that there were no significant differences in the percent diclofenac absorption, at least up to 3 hr ($p > 0.05$). This indicated that, once diclofenac has been released from the vehicle, the penetration rate through the stratum corneum appeared to be similar. Furthermore, comparison of the percent drug release *in vitro* and the percent drug absorbed *in vivo* indicated that the release rate of diclofenac was much slower than the absorption rate. This suggested that the release step of diclofenac from the vehicle be the rate-limiting step in the overall percutaneous absorption process. As a result, the difference in the initial amount of diclofenac found in the skin at time zero could be due to the difference in the release characteristics rather than the penetration through the skin.

13. Test of zero correlation was further applied to confirm if there were any significant correlations among the *in vitro* and *in vivo* parameters. The results in Table 20 indicated that only the *in vitro* parameters from the cellulose acetate membrane significantly correlated with the amount of drug found in the skin ($p < 0.05$). Durapore membrane and shed snake skin did not show any significant correlations. These findings were also in line with the different ranking results after the Duncan's test.

It was found that the release rate through the cellulose acetate membrane significantly correlated with the amount of diclofenac found in the

skin at all time points during the first three hours. However, the cumulative amount released after 6 hr from the same membrane was found to correlate with the *in vivo* data only at 1 and 3 hr. However, it can be concluded that there were general correlations between the *in vivo* percutaneous absorption of diclofenac based on the skin stripping technique and the *in vitro* release parameters from the cellulose acetate membrane. As a result, it should be possible to use these *in vitro* parameters as a rough indicator in predicting the *in vivo* percutaneous absorption of topical diclofenac gel products or to screen for the best formulation during product development.

14. By comparing the area under the amount of diclofenac found in the stratum corneum versus times (AUC_{0-6}) between the test (products B, C and D) and the reference product (A), the relative topical bioavailability could be calculated. Based on the data in Tables 24,only product C was bioequivalent to product A, with the relative bioavailability in the range of 126.41 %. On the other hand, products B and D were significantly less bioavailable than products A and C, with the relative topical bioavailability of only 27.63 % and 34.96 %,respectively ($p < 0.05$).*

15. In summary, the application of the *in vivo* skin stripping technique is a simple, rapid and economical method to evaluate the percutaneous absorption and bioavailability of topical NSAIDs such as diclofenac diethylammonium. The technique employs no blood withdrawal procedures necessary in the conventional systemic bioavailability study. Patient compliance is good and ethical problems are minimized due to its non-invasive approach. It thus appears to be a very convenient technique for rapid screening of drug formulations for their *in vivo* percutaneous absorption performance, especially during the drug development program.