CHAPTER V

CONCLUSION

The results from this study can be summarized as follows:

1. The six piroxicam gel products (A, B, C, D, E and F) contained equivalent amount of the active ingredient, with the percent labeled amount falling in the generally acceptable range of 90.0-110.0 % for all products.

2. The *in vitro* diffusion experiments employing the modified Franz diffusion cell apparatus indicated that the synthetic porous membrane such as cellulose acetate membrane 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 piroxicam gel products.

3. The amount of piroxicam in the receiver compartment of the diffusion apparatus was analyzed by a specific HPLC technique employing tenoxicam 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 six products tested with respect to the cumulative amount released at 6 hr. Duncan's test was further applied to rank these six products which can be roughly classified into four groups, i.e. the first group with the greatest (B), the second group with the great (C and F), the third group with the moderate (D and E), and the last group with the smallest amount of drug release (A).

5. ANOVA also showed significant difference in the release rate among the six products and Duncan's test can divide them into three groups. Product B was the fastest whereas products C and F were intermediate and products A, D and E were the slowest release products. Products B, C and D were subsequently selected as a representative from the three groups with different release rate (fast, intermediate and slow) for further studies and the results were then compared with product A, a reference piroxicam gel product.

6. The new born pig skin membrane was selected as a model membrane for the *in vitro* permeation experiments on the basis of their resemblance to the human skin. Permeation profiles were obtained by plotting the cumulative amount of drug per unit area (cm^2) 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 membrane. This indicated that, in contrast to the porous membranes, the new born pig skin possessed a significant barrier property which substantially retarded the penetration of piroxicam.

ANOVA results also showed that there were significant differences in both the amount of piroxicam 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 membrane data. Two processes were occurring simultaneously during the percutaneous absorption of piroxicam through the new born pig 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.

7. The technique was carried out to evaluate the *in vivo* local anti-inflammatory activities of topical piroxicam gel products using oxazolone induced ear edema. It can be seen that the greater the suppression of the ear edema, the smaller the difference in the weight between the right and left ears. Thus, the most effective product should be able to decrease the weight of the edema-plagued right ear as close to the normal weight of the left ear

as possible. The percent reduction of edema was calculated to be 49.6 to 57.5%, comparing to the control group.

Comparison of this parameter using ANOVA revealed that there was a significant difference ($p < 0.05$) among the four products and the control group. Duncan's test further revealed that the four products were significantly effective over control. However, they were not significantly different among each other.

8. Measurement of vascular permeability changes induced by histamine was another *in vivo* local anti-inflammatory activities experiment. It was found that the smaller the size of the blue spot, the greater the inhibition of permeability exerted by the products. The percent inhibition of vascular permeability was calculated to be 6.38 to 18.75%, comparing to the control group.

ANOVA results also showed that there was significant difference in the size of the blue spots ($p < 0.05$). Further Duncan's test revealed that the four products (and control) could be roughly classified into three groups, i.e. the most effective group (A, D and B), the intermediately effective group (C, A and D) and the least effective group (control and C). However, the Duncan's test result made it difficult to clearly distinguish among the four products and the control group. Therefore, another post-ANOVA test, the Dunnett's test was applied to compare the efficacy of each product relative to

only the control. It can be concluded that all products A, B, C and D showed a significantly better effect in the inhibition of vascular permeability than the control ($p < 0.05$).

9. The *in vivo* skin stripping technique was applied in the evaluation of the four piroxicam gel products for their *in vivo* percutaneous absorption and topical bioavailability. This 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 two week-washout period. The treatment sequence was randomized and the left forearm was applied with the gel under occlusion for 3 hr. After occlusion, the stratum corneum was removed by a series of adhesive tape to determine the amount of piroxicam remaining in this layer as a function of time for 6 hr. Loss of piroxicam from the stratum corneum during this period should indicate percutaneous absorption of the drug to deeper skin layers and underlying tissues.

10. Statistical analysis of the amount of piroxicam found in the stratum corneum (Randomized blocks ANOVA and Duncan's test) indicated that there was significant difference among the four products at time zero ($p < 0.05$). However, there were no significant differences among the four products at 1, 3 and 6 hr ($p > 0.05$).

11. The percent drug percutaneously 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 was no significant difference among the four products with respect to the percent absorbed at 1 hr ($p > 0.05$). At 3 and 6 hr, however it was found to be significantly different ($p < 0.05$). At 1 hr after termination of treatment, the percent loss of drug from stratum corneum was in the range of 22.79 to 45.04% for the four products. After 3 hr more piroxicam had permeated and the percent penetration of four products was in the range of 29.88 to 71.72 %. At 6 hr, there was about 41.78 to 78.10 % of piroxicam penetration.

12. The apparent first order rate constants for the percutaneous absorption process of the four piroxicam gel products were calculated from the plots of the natural log of the amount of piroxicam in the stratum corneum at various times. The plots were linear and the slopes of such plots gave the apparent first order rate constants of percutaneous absorption over the 6 hr.

Randomized block ANOVA and Duncan's test indicated that there were significant differences among the four gel products with respect to the first order percutaneous absorption rate constants over 6 hr ($p < 0.05$). This showed that the loss of drug from the stratum corneum appeared not to follow the same kinetics among the four products. Differences in the percutaneous absorption kinetics suggest that it may be unrelevant to directly

compare the extent of percutaneous absorption at various times among these products. In this case, the percent absorbed at different times may better represent the topical bioavailability, particularly with respect to the rate of absorption.

13. Test of zero correlation was further applied to establish the possible *in vitro-in vivo* correlations. Results in Table 22 indicated that there were no correlation between the *in vitro* parameters (release rate, permeation flux, cumulative amount released and cumulative amount permeated) and the in vivo skin stripping parameters (piroxicam content in the stratum corneum at time 0, 1, 3 and 6 hr, apparent first order rate constants, percent absorbed at 1, 3 and 6 hr, and area under the piroxicam content in the stratum corneum versus time curve ($AUC_{0\text{-}6hr}$)). In addition, there were no correlations between the *in vitro* release/permeation parameters and the *in vivo* pharmacological response parameters (i.e. ear weight difference and size of blue spots). Since the results from the *in vitro* experiments are usually employed to predict the *in vivo* performance of the products provided that significant correlations can be established, lack of the *in vitro-in vivo* correlations observed here thus indicated that the *in vitro* release/permeation models described in this study appear to have limited predictive value and may not represent the reliable *in vitro* models for evaluation of topical piroxicam gel products.

14. Test of zero correlation was further applied to establish possible correlations between the *in vivo* skin stripping and the *in vivo* pharmacological studies (Table 27). There were no correlations between the amount of piroxicam detected in the stratum corneum at various times and the two pharmacological parameters under study, i.e. the ear weight difference and the size of blue spot ($p > 0.05$). Other *in vivo* skin stripping parameters such as the rate constants and percent absorbed at 1, 3 and 6 hr also did not correlate with the ear weight difference. However, there were significant correlations observed between the percent piroxicam percutaneously absorbed at 1 and 3 hr and the size of blue spot following intravenous injection of pontamine sky blue into the rats ($r = 0.9940$ and 0.9502; $p < 0.05$). The percent drug absorbed at 1 hr gave the best correlation results ($r = 0.9940$). This parameter is equivalent to the initial rate of percutaneous absorption. The percent drug absorbed at 3 hr gave smaller correlation coefficient ($r = 0.9502$) which was still significant although rather marginally. The observation of the smaller correlation coefficient value at later times might be explained by the profile of the drug loss from the stratum corneum which appeared to reach saturation during 3-6 hr.

15. By comparing the percent absorbed of piroxicam through stratum corneum at 1 and 3 hr between the test products (B, C and D) and the reference product (A), the relative topical bioavailability could be calculated. All four products were not significantly different with respect to

the relative bioavailability at 1 hr. However, at 3 hr all test products were significantly different in the relative topical bioavailability; 164.53 (B), 131.41(D) and 68.55% (C) ($p < 0.05$), when compared to product A (100.0%). The four products could be statistically classified into three group, i.e. the most bioavailable group (B), the intermediate group (A and D), and the least bioavailable group (C).

Comparing the ability to reduce the size of blue spots between the test products (B, C and D) and the reference product (A), the relative topical bioavailability could be calculated. All the test products showed significant difference in relative topical bioavailability; 135.5 (B), 120.7 (D) and 46.1% (C) ($p < 0.05$), when compared to product A (100.0%). The four products could be statistically classified into three groups similar to the grouping of the percent absorbed at 3 hr, i.e. the most bioavailable group (B), the intermediate group (A and D), and the least bioavailable group (C).

16. 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 piroxicam. The technique employs no blood withdrawal procedures necessary in the conventional systemic bioavailability study and it does not use experimental animals for determining the pharmacological activities of topical products. 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.