Chapter III

Experimental Methodology

Material

Chemical :

Diclofenac diethylammonium, with supplement of Siam Pharmaceutical

Phenylbutazone, with the supplement of S. Co. Pharma Ltd.

Product A = Voltaren® Emulgel Lot No. 283 (Mfg. date. 30/12/93)

Product B = Locally made commercial product Lot No. CR525 (Mfg.date 1/12/35)

Product C = Locally made commercial product Lot No.50 XH009 (Mfg.date 28/8/34)

Product D = Experimental product (with a formula in Appendix I)

Methanol HPLC grade., Batch No. 9093-03

Disodium hydrogen phosphate AR., Batch No. 260632

Phosphoric acid

Sodium chloride AR., Batch No. 13445704, E.Merck.

All chemical were analytical or pharmaceutical grades and were used as received

Membranes:

: Cellulose acetate membrane , 0.45 μm pore size, 0.25 mm thickness , Millipore , USA.

: Durapore® membrane , 0.45 μm pore size , 0.16 mm thickness , Millipore , USA

: Shed snake skin (*Elaphe obsoleta*) kindly donated by PATA Zoo, Bangkok.

Equipments.

Modified Franz Diffusion cells and Diffusion Apparatus. With the supplement of Atlantic Pharmaceutical Co., Ltd.

UV Spectrophotometer , Spectronic 2000 , Bausch & lomb , USA.

pH Meter, Model SA 520 , Orion , USA.

Analytical Balance , Satorius GMPH , Range 300g/mg , Germany.

Thermostatted Shaker Bath, Julabovsw 1 , Juchheim Labortechnik HG , West Germany.

Vortex mixer , Scientific Industries. Inc.

Ultrasonic Bath, Bransonic 221, Branson, Smithkline

Company, USA.

High Performance Liquid Chromatography (HPLC) equipped with

a turnable absorbance detector, Water 484 Model M 484, Serial No.484-PRA 902 , USA.

an injector, Rheodyne injector 20 μl

a constant flow pump, Water 510 HPLC pump, Millipore USA.

an integrator, Water 745B Data Modules,Selrial No. 7BE/400678

a μ-Bondapak C18, 10 μm irregular, stainless steel column, 30 cm × 3.9 mm, ID., Water Assoc., USA.

Methods

Experiments were separated into two parts:

1. *In vitro* evaluation with respect to percent labelled amount, drug release and membrane permeation characteristics.

2. *In vivo* evaluation of percutaneous absorption using skin stripping technique

I. *In Vitro* Evaluation

The first part of the experiments constituted the *in vitro* evaluation of diclofenac diethylammonium topical preparations. The tests included the assay of percent labelled amount and the *in vitro* release/permeation studies using Franz diffusion cells. Three products were selected for this purpose based on their commercial availability and popularity, namely Voltaren® Emulgel (Ciba-Geigy) = product A, product B and C are locally made commercial products. Their lot numbers and date of manufacture are described in the Materials section. In addition, one experimental emulgel product (product D) was specially prepared for this study and included for comparison. Its formulation and method of preparation are given in Appendix I. All the four products contained the same amount of the active drug, i.e. 1.16 % of diclofenac diethylammonium equivalent to 1 % diclofenac in suitable gel bases.

1. 1 Assay of Percent Labelled Amount

Prior to the *in vitro* release/permeation studies, the products were assayed for their contents of diclofenac diethylammonium. The acceptable assay limit was 90.0 - 105.0 % labelled amount.

1.1.1 Preparation of Standard Solution

Standard solution was prepared by accurately weighing about 23 mg of diclofenac diethylammonium in a 100 ml volumetric flask. Sixty ml of 0.01 N sodium hydroxide was added and the flask was shaken until the drug was completely dissolved. The solution was then adjusted to volume with 0.01 N sodium hydroxide. The concentration of the stock solution was 0.23 mg/ml. Ten ml of this solution was pipetted into another 100 ml volumetric flask and adjusted to volume with 0.01 N sodium hydroxide , the final concentration of the standard solution being 23 μg/ml.

1.1.2 Preparation of Sample Solutions

Approximately 2 gm of each of the diclofenac gel products were accurately weighed in a 100 ml volumetric flask. Sixty ml of 0.01 N sodium hydroxide was added to the flask which was subsequently shaken until the drug was dissolved. The flask was adjusted to volume with 0.01 N sodium hydroxide. The solution was then filtered through Whatman paper No.1, the first 10 ml being discarded. Each product was prepared in duplicate. Ten ml of the filtrate was pipetted to a 250 ml separating funnel. 20 ml of 0.1 N hydrochloric acid was added and the solution was extracted with two 20 ml portions of chloroform, followed by another two 30 ml portions. The chloroform extracts were combined and washed with three 30 ml portions of

distilled water. The extracts were then filtered through Whatman paper no.1 and back-extracted with three 30 ml portions of 0.1 N sodium hydroxide. The alkali extracts were combined in a 100 mlvolumetric flask and adjusted to volume with 0.1 N sodium hydroxide. The extracts were analyzed with UV spectrophotometer at a wavelength of 276 nm using 0.1 N sodium hydroxide as blank. By comparing the absorbance with that of the standard solution, the percent labelled amount of diclofenac diethylammonium in each gel products was calculated.

1.2 *In Vitro* Release/Permeation Studies

After determining their content, the four products were subjected to the *in vitro* tests using a modified Franz diffusion apparatus. The apparatus consisted of 4 water-jacketed cell holders fixed on the same mounting case with built-in magnetic stirrer,which custom-designed by department of mecanical engineer Chulalongkorn university (Figure 5). The diffusion cells were locally manufactured with the design slightly modified from the Franz diffusion cell. Each cell consisted of two parts, namely the donor and the receiver compartments vertically attached to each other via a metal clamp. The inside diameter was 1.4 cm, equivalent to the area of 1.54 cm^2. The receptor volume in each cell ranged from 5.7 to 6.1 ml. The diagram of the apparatus is shown in Figure 6. The two compartments were separated by a thin membrane barrier. Three types of membranes were selected for use in this study. These were cellulose acetate membrane, Durapore® and shed snake skin.

Briefly, 1.5 gm of each gel preparation was placed in the donor chamber on top of the receiver compartment which contained pH 7.4, isotonic

Figure 5: Photograph of Franz diffusion cell

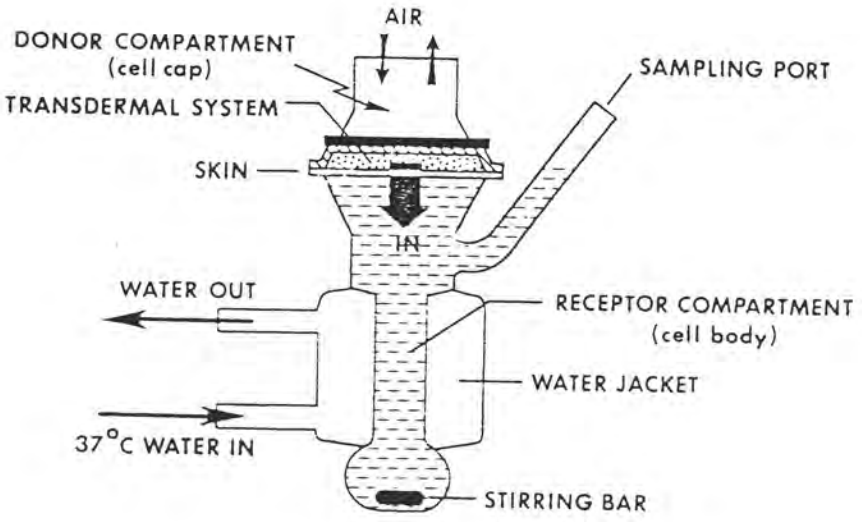


Figure 6: Diagramatic of the Franz diffusion cell

phosphate buffer solution. The preparation of the buffer is described in 1.2.2. The system was maintained at 37°C throughout the study by means of circulating water bath. The steady-state flux and the cumulative amount of diclofenac which was released from the product or penetrated through the membrane were then calculated following the analysis of the drug in the receptor fluid at various times.

1.2.1 Preparation and Pretreatment of Membranes

Cellulose acetate membranes. Cellulose acetate sheets were washed out and rinsed with distilled water before immersing in the pH 7.4 isotonic phosphate buffer for about six hours prior to use. After pretreatment, the membrane sheet was mounted directly onto the diffusion apparatus.

Durapore® membranes. Durapore® sheets were cut at the same size as the diffusion cell diameter and rinsed with distilled water before immersed in pH 7.4 isotonic buffer about six hour before mounted onto the apparatus.

Shed snake skin. Shed snake skin specimens from *Elaphe obsoleta* were selected as representatives of stratum corneum, the major barrier to percutaneous drug absorption. It was kept in refregerator at 4°C Before using,it was thawed in room temperature and the dorsal part of the specimen was cut about the same size as the diffusion cell and then immersed in pH 7.4 isotonic phosphate buffer for 12 hours prior to use.

1.2.2 Preparation of pH 7.4, Isotonic Phosphate Buffer

1.9 gm of monobasic potassium phosphate was dissolved in 400 ml of distilled water. 4.11 gm of sodium chloride was then added to this solution. 8.1 gm of dibasic sodium phosphate was separately dissolved in another 400 ml of distilled water. The two solutions were then mixed together and the final volume was adjusted to 1,000.0 ml with distilled water. Adjust the pH to 7.4 $\pm$ 0.02 with either 1 M phosphoric acid or 1 N sodium hydroxide.

1.2.3 Diffusion Studies

A membrane which had been previously pretreated was mounted between the donor and receiver compartments by means of a clamp. The membrane was placed in such a way that the upper part was in contact with the preparation in the donor chamber and the the other side with the receiver fluid. Excess amount of diclofenac gel products (1.5 gm) were applied over the membrane surface in the donor chamber. A small magnetic bar was placed in the receptor compartment. Isotonic phosphate buffer pH 7.4, prewarmed at 37 °C, was filled into the receiver compartment, the volume varying from 5.7 to 6.1 ml depending on the calibrated volume of each diffusion cell.

The experiment was then started by turning on the magnetic stirrer which rotated at 300 $\pm$ 5 rpm. The temperature of the assembled diffusion cell was maintained at 37°C by means of circulating water jacket connected to a constant temperature water bath. Care was taken during the cell assembly that no bubble were observed underneath the membrane.

All the sampled receptor fluids were analyzed using the HPLC technique. The diclofenac diethylammonium concentrations in the receptor compartment were determined from the calibration curve. The amount released or permeating through the membrane was calculated by multiplying the drug concentration with the respective receptor volume (5.7 to 6.1 ml). The cumulative amount of the drug found in the receptor compartment at various time intervals was then plotted as a function of the sampling time. The steady-state flux for the release/permeation was subsequently calculated from the slope of the linear portion of each plot. Analysis of variance (ANOVA) was then applied at 5 % significant level to see if there were any significant differences in cumulative amount and steady-state flux among the four products. If such differences existed, further statistical analysis was performed in order to rank the products using tests such as Duncan's test.

1.2.4 HPLC Analysis

The high performance liquid chromatographic system consisted of a constant flow pump (Waters 510, Millipore, Massachusetts, USA), a wavelength-adjustable ultraviolet detector (Waters Model M484) and an integrator (Waters 745B data module). Briefly 1 ml of the sampled fluid was mixed with 1 ml of phenylbutazone internal standard solution (conc 4 mg/ml) prior to injection. The analytical procedures and chromatographic conditions are as follows:

1.2.4.1 Preparation of Phenylbutazone Internal Standard Solution

About 20 mg of phenylbutazone was accurately weighed in a 100 ml volumetric flask followed by addition of 20 ml methanol. The flask

was shaken until the compound completely dissolved and then adjusted to volume with methanol. One ml of the solution was pipetted to a 50 ml volumetric flask and diluted to volume with methanol, the concentration of phenylbutazone being 4 μg/ml.

1.2.4.2 Preparation of Diclofenac Diethylammonium Standard Solutions

About 40 mg of diclofenac diethylammonium was accurately weighed in a 10 ml volumetric flask. Five ml of methanol was added to dissolve the drug. After dissolution, the flask was adjusted to volume with the solvent. One ml was pipetted into a 100 ml volumetric flask and further diluted to volume with pH 3.3, 0.05 M phosphate buffer , the final concentration of this standard stock solution being 40 μg/ml. 0.125, 0.25, 0.5, 1.0, 3.0, 4.0 and 5.0 ml of the standard stock solution were separately pipetted and transferred into seven 10 ml volumetric flasks, each containing 5.0 ml of the internal standard solution. All the flasks were subsequently diluted to volume with pH 3.3, 0.05 M phosphate buffer so that the final drug concentrations were 0.5, 1.0, 2.0, 4.0, 12.0, 16.0 and 20.0 μg/ml, respectively. The final concentration of phenylbutazone internal standard was equal to 2.0 μg/ml in all solutions.

1.2.4.3 Preparation of Sample Solutions

One ml of the receptor fluid sample was pipetted and mixed with one ml of internal standard solution. Thus, the final concentration of the internal standard prior to HPLC analysis was kept constant at 2.0 μg/ml in both the standard and sample solutions.

1.2.4.4 Preparation of pH 3.3, 0.05 M monobasic sodium phosphate buffer

7.05 gm of anhydrous monosodium dihydrogen phosphate was dissolved in 980 ml distilled water. The pH of the solution was adjusted to 3.3 with dropwise addition of 1 M phosphoric acid. The final volume was subsequently adjusted to 1,000 ml with distilled water.

1.2.4.5 HPLC conditions for Diclofenac Diethylammonium Analysis

The conditions for the analysis can be summarized as follows:

Column	:	μ-Bondapak C_{18} (Waters, Massachusetts, USA)
Mobile phase	:	A mixture of methanol and pH 3.3, 0.05 monobasic sodium phosphate, 75: 25 %v/v
Detection wavelength:		280 nm
Flow rate	:	1 ml/min
Attenuation	:	8-16
Chart speed	:	0.25 cm/min

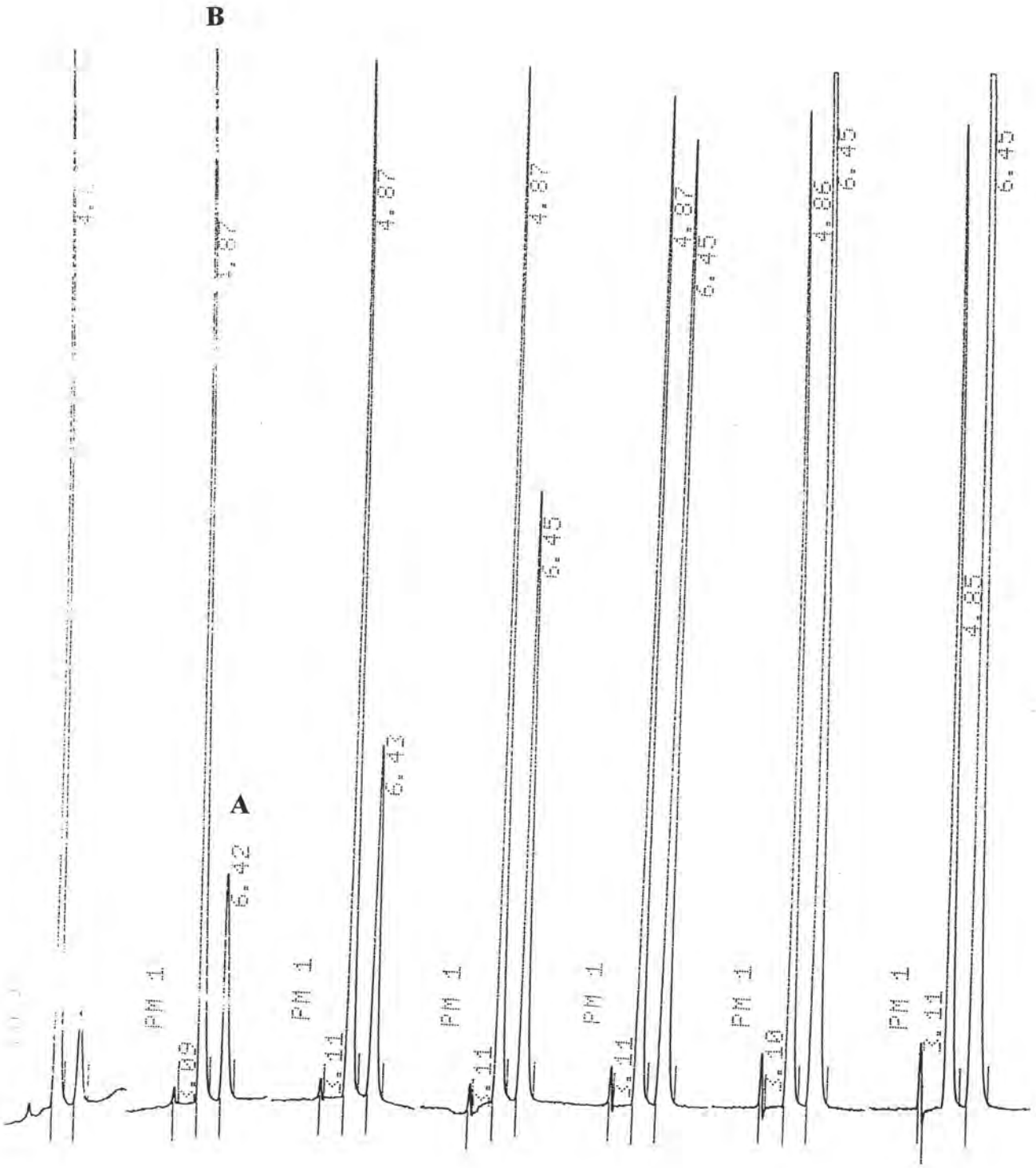


Figure 7: Chromatogram and standard curve of diclofenac diethylammonium (A = diclofenac diethylammonium , B =Internal standard or Phenylbutazone)

Injection volume	:	20 μl
Internal standard	:	2.0 μg/ml final conc. of phenylbutazone
Retention times	:	Phenylbutazone 4.7 min
		Diclofenac diethylammonium 6.3 min

The representative chromatograms of the standard and sample solutions, together with the standard curve are shown in Figure 7

II. *In Vivo* Evaluation of Percutaneous Absorption Using Skin Stripping Technique

After the *in vitro* tests, the four gel products were subjected to the *in vivo* evaluation in humans. The technique was based on the analysis of drug remaining in the stratum corneum after topical application of the product. Caron et al (1991) were the first to use this technique to quantitatively assess the topical bioavailability of hydrocortisone creams . They also found a good correlation between the amount of hydrocortisone remaining in the stratum corneum and the degree of skin blanching produced by the vasoconstriction effect of the steroid.

Following application of the product to the designated area (e.g. mid forearm), the stratum corneum was sequentially removed from the skin surface by means of adhesive tape strips. Each skin stripping by a piece of tape resulted in a certain amount of stratum corneum being removed. Analysis of drug in the tape-stripped stratum corneum reflected the amount of drug that was released and permeated through the upper layers of the skin. Topical bioavailability was thus estimated from the amount of drug found in the tape stripped stratum corneum following spicific period of drug application. The

greater the initial amount found after administration, the higher the extent drug release and penetrate.These would be ,therefore reflect the higher degree of percutaneous absorption and topical bioavailability.

This part of study attempted to apply the skin stripping technique to another class of drugs, namely the NSAIDs with diclofenac chosen as the model drug. The technique was also further modified to include analyses of the drug remaining in the stratum corneum at different times. Disappearance of the drug from this layer as the time elapsed should confirm the occurrence of percutaneous drug absorption. This should also permit the evaluation of its absorption kinetics as well as the percent drug absorbed per unit time.

2.1 Preliminary Study

The purpose of the preliminary study was to determine the optimum conditions of the experiments, particularly with respect to: 1) the appropriate skin stripping sequence and the optimum occlusion time after drug application and 2) the possible difference in skin stripping results due to difference in application sites (right or left forearm).

2.1.1 Determination of Appropriate Skin Stripping Sequence and Optimum Occlusion Time

This part of the experiments determined an appropriate skin stripping sequence for each application spot. It also determined the appropriate period of skin occlusion following drug application before skin stripping took place. Three healthy volunteers participated in this study. All of them did not take diclofenac or any other drugs at least one week before entering the study.

Only the innovator's product, Voltaren® emulgel, was used. By means of a paper template with nine punched holes, the area of their left mid-forearms was marked by pen into corresponding nine small squares, each having 1 x 1 cm dimension. These squares are called the application spots on which the product was applied. The 3 x 3 arrangement of the nine application spots is shown in Figure 8 each spot being separated by a distance of 1.5 cm.

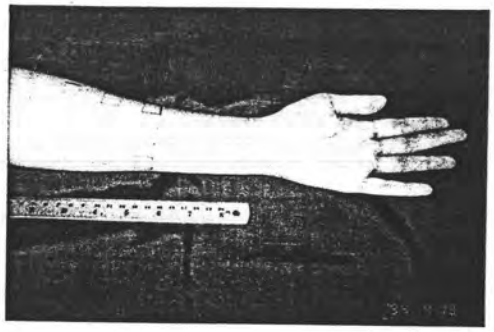
Through the hole in the template, the same excessive dose (about 100 mg) of Voltaren® was subsequently smeared on each of the designated nine spots using a small wire. Care was taken to make sure that the same amount of the gel was applied to each spot and that the gel contained within the spot boundary. All the spots were then occluded with Tegaderm® tape for different periods of time depending on their position in the 3 x 3 arrangement. Such the arrangement allowed the application spots to be divided into three parallel rows, namely, upper, middle and lower rows, each containing 3 consecutive spots. The occlusion period after drug application was arbitrarily set at 1, 3 and 6 hours and was randomly assigned to each row. The purpose for the random assignment of the occlusion period was to balance out any difference due to the row effect so that the effect of the occlusion time, if existed, could be clearly seen. After the specified occlusion period, the Tegaderm® coverings were removed from each row and any residual gel was gently wiped out three times from the surface of each spot using cotton buds. After removal of the excess gel, the skin stripping process was then started.

Ten pieces (or strips) of Transpore® tape, cut to about the same size as the application spots (1 x 1 cm), was consecutively placed on an application spot with the use of small forceps. After placing the first piece, a steady pressure was applied to the application spot by means of a small roller. After a

single roll, the tape was removed from the application spot and placed in a 13 x 100 mm culture tube. This would complete one stripping. Each piece of tape was used to strip the skin only once. Following the first stripping, the same procedure was repeated nine more times on the same application spot. Previous experiments (Pershing, 1992 ; Rougier et al,1990) have shown that ten strippings per spot were adequate to remove most of the stratum corneum.

To determine the appropriate number of the sequential tape strips to be used in the actual drug analysis, the ten strips were separately analyzed for diclofenac content by pairing together the first and second strips, third and fourth, fifth and sixth, seventh and eighth, and the ninth and tenth strips, making a total of five pairs. Therefore, during the skin stripping of each application spot, five culture tubes were needed to keep the respective five pairs of tape strips. Analysis of the drug in each of the sequential pairs would allow one to determine the appropriate number of tape strips that would be combined for accurate analysis of diclofenac in the stratum corneum during the next phase of the study. An HPLC technique was used in all the skin stripping studies to analyze the amount of drug in the tape-stripped stratum corneum. Briefly, each pair of tape strips was immersed in 2.0 ml of the mobile phase containing 2 μg/ml of phenylbutazone as the internal standard. After vortexing for one minute, the mixture was centrifuged at 3,000 rpm for 5 minutes. The clear supernatant was then injected onto the HPLC. Details of the analytical conditions were similar to the *in vitro* tests as described in 1.2.4.5.

The average amount of diclofenac diethylammonium found in each pair was then plotted against the sequential pair number (No. 1 to 5) for each of the three rows in each subject. The reason for making these plots was to see if there was any excess drug that still remained on top of the stratum corneum. If there existed, analysis of the drug in the first few strips should give the drug

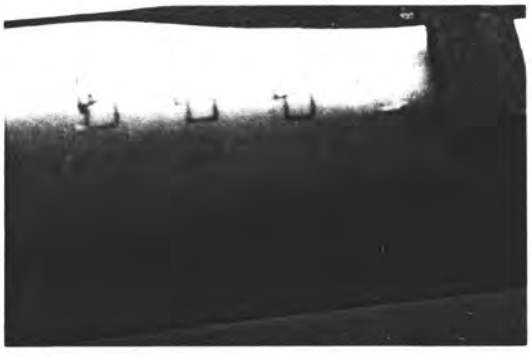


a. The area was marked by pen into 3×3 arrangement (each of spot was 1 cm^2).

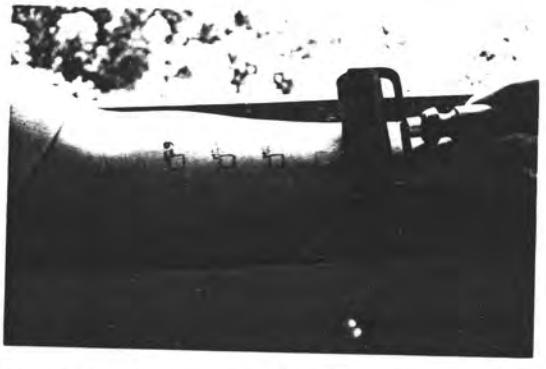
b. The excessive dose of voltaren® was smear l on each spot.

c. At appropriate time (1,3 or 6 hr) , the covering were removed.

d. The residual gel was wiped out by cotton buds



e. Ten strips of Tegaderm® were placed on the application spot.



f. Steady pressure was applied by means of a small r

g. Transpore® tape was removed with a small forcep.

Figure 8: Experimental steps for testing the appropriate skin stripping sequence and optimum occlusion time

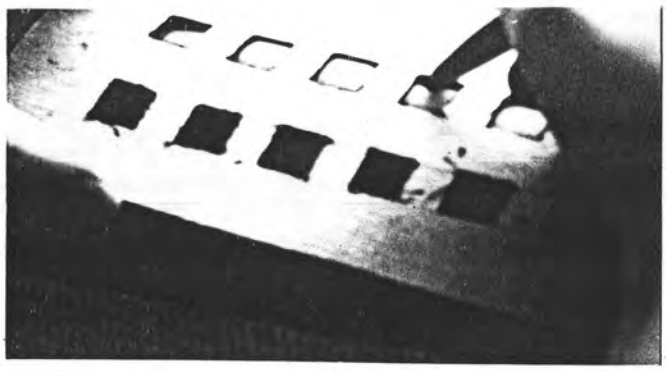
content higher than other subsequent strips. As a result, these initial strips should not be used for analysis since the drug found would not represent the amount of drug in the stratum corneum. After identifying the number of the first few strips that removed the excess drug from the surface, the remaining tape strips could be combined for subsequent drug analysis. Thus, the amount of drug found in later strips should represent the drug that actually permeated through the stratum corneum.

Comparison was then made between the average amounts of drug found in the tape-stripped stratum corneum after different occlusion periods in the three subjects. following occlusion each spot was sequentially tape-stripped for ten times, the first few strips being discarded. The exact number to be discarded also depended on the results of the previous experiment. The remaining strips were then combined in a culture tube for subsequent HPLC analysis. The result of this study would determine the optimum application time that would be used in the subsequent experiments.

2.1.2 Effect of Difference in Application Sites

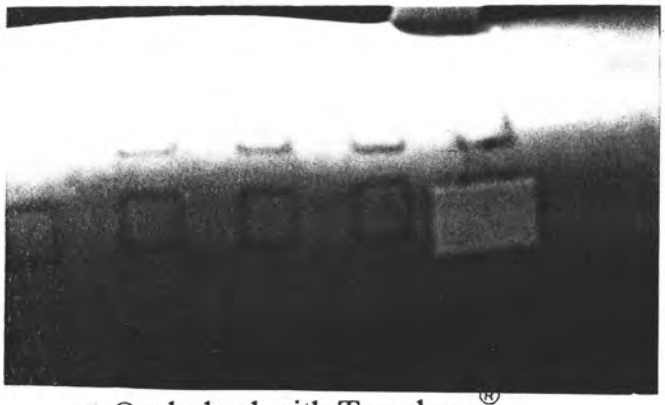
The next phase of study constituted the determination of the effect of different application sites on the amount of drug found in the stratum corneum, i.e. whether there were any differences due to the row effect within the same forearm or any differences between the left and right forearms of the same subject.

This study was carried out using one healthy volunteer who had not taken diclofenac or any another drugs at least one week before entering the study. As with the above experiments, Voltaren® emulgel was used as the test



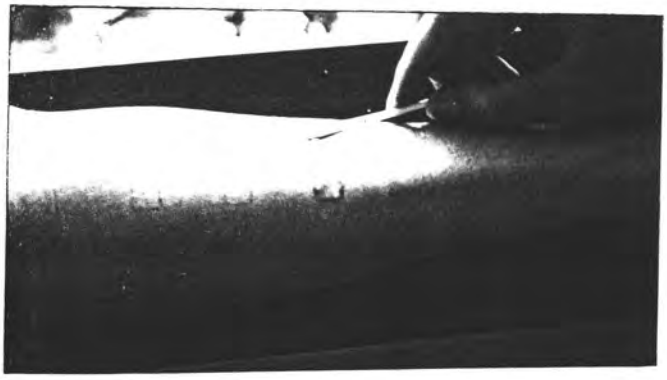
a. The application were five paralleled rows

b. The excessive dose of voltaren® was smeared on each spot.



c. Occludeed with Tegaderm®

d. At appropriate time (1,3 or 6 hr), the covering were removed.



e. The residual gel was wiped out by cotton buds.

f. Ten strips of Transpore® were placed on the application spot.

g. Steady pressure was applied by means of a small roller.

h. Transpore tape was removed with a smoall forcep.

Figure 9: Experimental steps for testing the effect of difference in application sites

product. The arrangement of the application spots in each forearm was slightly different from 2.1.1 in that there were five parallel rows instead of three, each containing three spots as shown in Figure 9 Thus, a total of fifteen application spots were marked on each forearm, the first row being about 3 cm below the antecubital fossa and the fifth (lowest) row about 3 cm above the wrist. Each square-shaped spot had a 1 x 1 cm dimension similar to 2.1.1. Marking of the application spots as well as drug administration were also facilitated by means of a special paper template correspondingly cut to contain fifteen square-shaped holes in a 5 x 3 arrangement. About 100 mg of Voltaren® was then applied on each spot in a manner similar to 2.1.1. When the administration was completed, all the spots were occluded with Tegaderm® tapes. After occlusion for a specified period of time (the exact occlusion time was determined from the experiment in 2.1.1), By comparing the average amounts of diclofenac found in each of the five rows, the effect of different rows within the same forearm could be delineated following the analysis of variance (ANOVA) at 5 % significant level. In addition, student's t- test, also at 5 % significant level, was applied to determine if there was any difference in the average amount of diclofenac found in the stratum corneum between the left and right forearms.

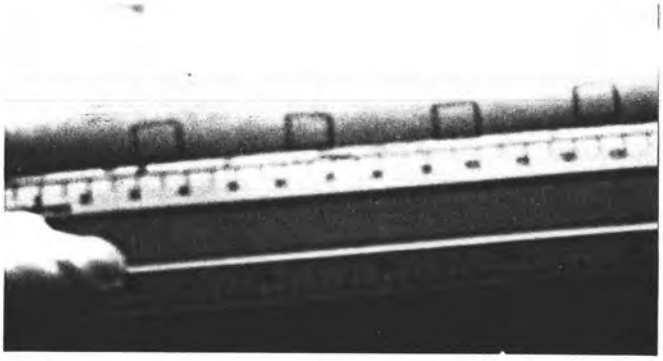
2.2 Evaluation of Percutaneous Absorption of Topical Diclofenac Gel Products Using *In Vivo* Skin Stripping Technique

After the preliminary study to determine the optimum *in vivo* testing conditions, the four diclofenac diethylammonium gel products were subjected

to the skin stripping test. The study was a single-blinded, completely randomized crossover design employing eight healthy volunteer all females with age ranging from 24 to 30 years (average 26.6± 1.8). All subjects had not taken any medications for at least one week before and during the study.

Each volunteer received a completely randomized sequence of the four products on four occasions, separated by a washout period of one week. Whether each product was applied to the left or right forearm was also randomized to average out any possible influence due to site difference. Similar to 2.1, a paper template was used to mark the application spots on the forearm and to facilitate the drug administration. However, there were fiour parallel rows in this arrangement, each row containing two spots. Thus, a total of eight application spots were used in this study. The position of the spots in the forearm was such that the top row was at least 3 cm below the antecubital fossa and the lowest (fourth) row about 3 cm above the wrist (Figure 10)

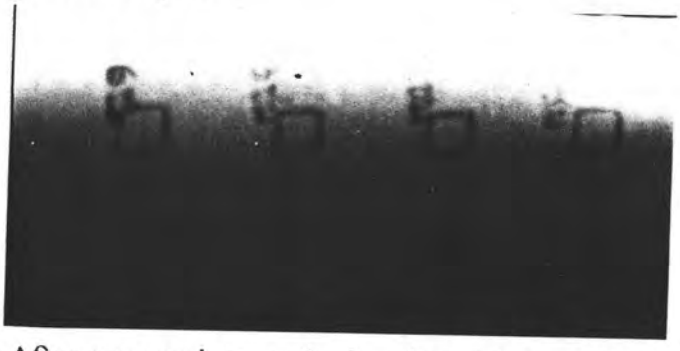
The same excessive dose (about 100 mg) of each product was applied on each of the eight spots. Immediately after drug administration, each row was covered with Tegaderm® tapes for an optimum period of time. After this occlusion period, Tegaderm® tapes were removed and all the spots were gently but thoroughly wiped out of any excess gels to terminate the drug administration. One row of spots was then randomly selected for skin stripping whereas the remaining three rows were covered again with new strip of Tegaderm®. The stripping procedure was the same as in 2.1 using ten strips of Transpore® tape with the first few strips being discarded. The amount of diclofenac found in this row was designated as the initial amount detected in



a. The application spots were 4 X 2 arrangment

b. The excessive dose of voltaren® was smeared on each spot.

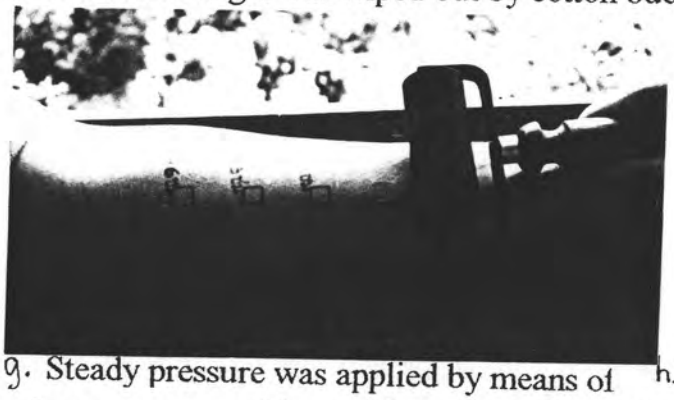
c. Occludeed with Tegaderm®



d. After appropriate occlusion time (1,3 or 6 hr) , the covering were removed.

e. The residual gel was wiped out by cotton buds.

f. Ten strips of Tegaderm were placed on the application spot.



g. Steady pressure was applied by means of a small roller.

h. Transpore® tape was removed with a smoall forcep

Figure 10: Experimental steps for evaluation of percutaneous absorption using skin-stripping technique.

the stratum corneum at time zero after termination of the drug administration. Another row was then randomly selected for stripping when one more hour had elapsed to determine the amount of drug remaining at one hour after termination of drug administration. Similarly, the remaining two rows were randomly stripped at 3 and 6 hours after termination of the drug administration to determine the amount of drug remaining in the stratum corneum after 3 and 6 hours, respectively.

The combined strips from each spot were immersed in 2.0 ml of mobile phase containing the internal standard and analyzed for diclofenac diethylammonium content by HPLC as in 2.1.2

The study would be terminated immediately if major pruritus, redness or burning sensation were observed at the site of application as a result of the allergic reactions to the drug or the gel components. After HPLC analysis, the amount of diclofenac found in the tape-stripped stratum corneum was then plotted as a function of time. The percent of drug percutaneously absorbed at various times was also calculated for each product for comparison within each subject using two-way ANOVA and an appropriate rank test at 5 % significant level (e.g. Duncan's test). Statistical comparison was also made with respect to the amount of drug found in the stratum corneum at different times between the four products.

2.3 Correlation Studies between *In Vitro* and *In Vivo* Data

Attempt was made to find any correlation between the *in vitro* release/permeation study results and the *in vivo* skin stripping data. First, the ranking relationship obtained from post-ANOVA multiple range test after the *in vitro* tests of the four products (i.e. the steady-state flux and cumulative amount) were compared with those of the *in vivo* results (i.e. the amount found in the stratum corneum and the percent of drug percutaneously absorbed at various times) to initially determine if there were any similarities in the ranking order among the four products. Second, the correlation test was applied at 5 % significant level to determine if there were any significant correlation between these *in vitro* and *in vivo* parameters. Results from this part of study would be indicative of the mechanism underlying the overall percutaneous absorption of diclofenac from these gel products. Furthermore, if there were any correlation existing between the *in vitro* results and the *in vivo* skin stripping data, an appropriate *in vitro* release or permeation test could be developed for use as a relatively simple and rapid technique to predict the *in vivo* percutaneous absorption and topical bioavailability of drugs such as diclofenac diethylammonium.