DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 17(1), 91-111 (1991)

THE EFFECTS OF pH AND CHEMICAL ENHANCERS ON THE PERCUTANEOUS ABSORPTION OF INDOMETHACIN

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ABSTRACT

The absorption of indomethacin through excised hairless mouse skin from aqueous solution was determined at different pH values. We found that the rate of its absorption increased with decreasing pH. Its distribution coefficient in octanol-phosphate buffer was also pH dependent. Furthermore, the change of permeability coefficient with pH correlated well with the distribution coefficient by a two-degree polynominal equation. The individual incorporation of five chemical enhancers into a polymeric patch at optimal pH resulted in an increase or decrease in the in vitro absorption rate and in the amount absorbed during the first 24 hours depending on the enhancer and its concentration used. Both sodium cholate at 4 and 6 %, and sodium lauryl sulfate

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at 4 % increased the absorption rate about four to seven times compared to the control. The in vivo absorption using rabbits from patch containing 6 % sodium cholate also showed an increase in rate and the AUC compared to that from the control; however, the extent of the increase was much less compared to that obtained from the in vitro study. The stability of this drug in aqueous solution was also studied as a function of pH. It was confirmed that indomethacin was more stable at lower pH values. The pH-rate constant profile also indicated a specific base catalysis for its degradation at pH above 6.5. Due to its small solubilities at lower pHs, an optimal pH near 5 was suggested for the preparation of a transdermal delivery system for indomethacin.

INTRODUCTION

Indomethacin is a non-steroidal drug with anti-inflammatory, antipyretic and analgesic properties (1). Upon oral administration, however, serious side effects such as headache, nausea, vomiting, diarrhoea and duodenal ulcer usually occur (2, 3). Several indomethacin preparations for oral use have been studied in an attempt to minimize these adverse effects (4, 5). No significant protection was demonstrated from these preparations (6, 7).

The delivery of drugs through the skin for systemic effects has attracted much attention in recent years. Drugs such as scopolamine (8), clonidine (9, 10) and nitroglycerin (11) have

all been shown to have better therapeutic effect when given transdermally than otherwise. The formulation of these so-called transdermal delivery systems requires, however, that the dose is small and that the rate-limiting barrier for the absorption resides in a control membrane within the device (12).

In our previous study (13), a polymeric patch containing indomethacin for transdermal delivery was prepared, and the drug release profile from this patch was characterized. The purpose of this study was to further examine the effects of pH and chemical enhancers on the performance of these patches as transdermal delivery systems for indomethacin. The chemical enhancers chosen for this study were azone, dimethyl sulfoxide, stearyl alcohol, sodium lauryl sulfate and sodium cholate. Both *in vitro* and *in vivo* methods for percutaneous absorption studies were followed.

METHODS

Materials

Indomethacin and glycerin (Sigma Chemical Company), polyvinyl alcohol (BF26, Chang Chung Chemical Co., Taiwan), monobasic sodium phosphate and dibasic sodium phosphate (E. Merck, Darmstadt, West Germany), dimethyl sulfoxide (Wako Pure Chemical Industries), sodium cholate (E. Merck, Darmstadt, West Germany), stearyl alcohol (Kanto Chemical Inc., Japan), sodium lauryl sulfate (Santoko Chemical Company, Osaka, Japan) and other chemicals used in this study were either reagent grade or USP

grade. Azone was a gift from Nelson Research and Development (Irvine, CA., USA).

Stability Study

A series of aqueous solutions containing 20 μg/mL of indomethacin were prepared with phosphate buffers at pH 5.5, 6.0, 6.5 and pH 7.5. Aliquots (1.0 mL) of these solutions were sealed in 1 mL ampules and incubated at 50°C (± 0.2) and 60°C (± 0.2). At specified time intervals after incubation, two ampules were withdrawn and temporarily stored at -20°C for assay. Samples were analyzed by high-performance liquid chromatography (HPLC) as described below.

Determination of Distribution Coefficient

The distribution coefficient of indomethacin was determined in octanol-phosphate buffer system at pH values of 5.51, 6.01, 6.51 and 6.74. The total phosphate concentration was kept at 0.2 M. Three different volume ratios of octanol to phosphate buffer (4:2, 6:2 and 6:1) were used for each determination. The concentration of indomethacin ranged from 30 to 300 μg/mL. Each solution was placed in a test tube with a screw cap lined with a telflon cushion. These test tubes were rotated for three hours at 35 ± 0.5°C and then centrifuged at 3,000 rpm for 5 min. The concentration of indomethacin in the aqueous layer was determined by the HPLC method. The distribution coefficient was calculated

according to the following equation (14):

$$DC = \frac{(C_a - C_b)\, V_w}{C_b\, V_o}$$

where DC is the distribution coefficient; C_a and C_b represent the drug concentration in the aqueous layer at the beginning and at equilibrium, respectively; V_w and V_o represent the volume of aqueous layer and octanol layer, respectively.

Preparation of Indomethacin Patches

Polymeric patches containing 2 % indomethacin and various amounts of dimethyl sulfoxide, stearyl alcohol, sodium lauryl sulfate, azone or sodium cholate were prepared as before (13). The water contents were reduced to compensate for the inclusion of these chemicals.

In Vitro Study

Franz diffusion cells were used for the determination of skin penetration of indomethacin from the patch. The skin specimen was prepared from the hairless mouse, weighing 25-30 gm, of both sexes. The mouse was sacrificed by the dislocation of the spinal cord at the neck. The full skin in the section of abdomen was excised and frozen (15).

Indomethacin aqueous solution were also prepared in phophate buffers at pH 5.0, 5.5, 6.0, 6.5 and 7.5 . Ten percent of polyethylene glycol 400 was added in each solution to increase

its solubility. The drug concentration was 200 μg/mL except at pH 5.0, for which 100 μg/mL was used due to lower solubility.

The donor-side of the Franz diffusion cell was fitted with the patch and filled with 1.8 mL of indomethacin aqueous solution. The receptor-side had a capacity of 6 mL and was filled with pH 7.4 phosphate buffer. A constant temperature was maintained at 35 ± 0.2°C. At each sampling interval, an aliquot of the solution on the receptor-side was drawn off and assayed by the HPLC method. The volume of the solution was kept constant by replacing the sample volume with equal amount of the buffer. Each preparation was studied in triplicate.

In Vivo Study

Eight Newzealand white rabbits weighing 2-3 Kg, of both sexes, were randomly divided into two groups, each group comprising of four rabbits. To one group was applied the indomethacin patches without the enhancer, while another group received the indomethacin patches containing 6 % of sodium cholate.

The rabbit was shaved on the back with an electrical clipper. Two pieces of indomethacin patches were placed at the shaved area and secured with a band tape and an elastic bandage. A #23 gauge needle was used to puncture the ear marginal vein at 7, 16, 19, 23, and 30 hours, and about 1.5 mL of blood sample was collected in a Venoject tube containing sodium heparin. Blood samples were centrifuged for ten minutes at 3,000 rpm. About 0.5

mL plasma sample was used in the assay. The extraction method was modified from the one reported by Tsai and Natio (16) as follows: 0.1 mL p-cresol solution with a concentration of 40 μg/mL was used as the internal standard, and additionally, 2 mL of pH 5 sodium citrate buffer, 0.05 M, was added. After mixing, the mixture was extracted with 5 mL of cyclohexane/ether (50:50) in a rotator set at 50 rpm for 40 minutes. The organic solvent in the upper layer was then withdrawn and evaporated in a vacuum system at 40 °C. The residue was dissolved in 0.25 mL of the mobile phase of the HPLC and its concentration was determined using the HPLC methodology.

HPLC Condition

The mobile phase was composed of acetonitrile/sodium phosphate buffer (pH 4.0, 0.05 M) with a ratio 40:60, The flow was 1.0 mL/min. A C18 μ-Bondapak column (stainless, 3.9mm (ID) x 30 cm) was used for the separation. The detector wavelength was set at 254 nm.

RESULTS

Stability Study

Figure 1 shows the first-order degradation of indomethacin at 50 °C and 60 °C in phosphate buffer at different pHs. The apparent first-order rate constants for the degradation of indomethacin were determined from the slopes and listed in Table 1.

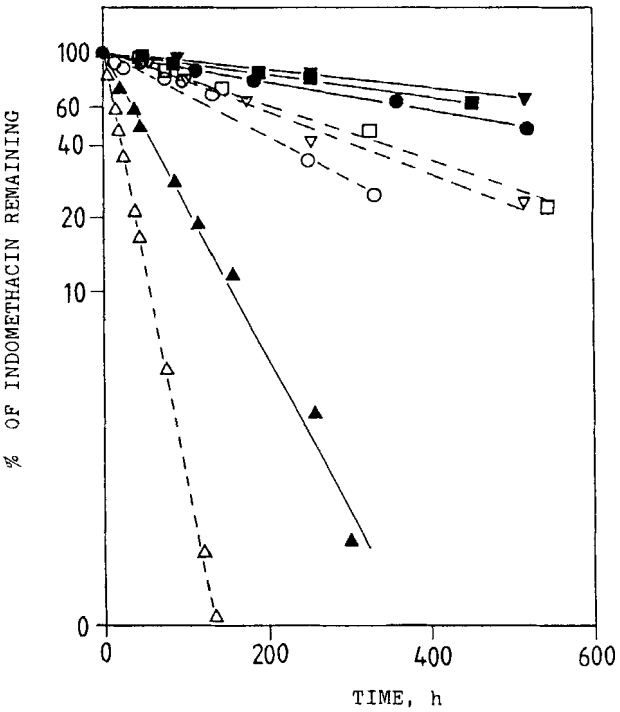

FIGURE 1

Indomethacin degradation at 50 °C and 60 °C in phosphate buffer at pH 7.5, 6.5, 6.0 and 5.5.
Keys: At 50°C: (-▲-), pH 7.5; (-●-), pH 6.5; (-■-), pH 6.0; (-▼-), pH 5.5.
At 60°C: (-△-), pH 7.5; (-○-), pH 6.5; (-▽-), pH 6.0; (-□-), pH 5.5.

The plots of log K_{obs} vs. pH for these two temperatures were shown in Figure 2.

Distribution Coefficient

Table 2 lists the determined distribution coefficients of indomethacin in octanol-phosphate buffer at different pHs. Double

TABLE 1

The Apparent Frist Order Degradation Constants of Indomethacin at Different Temperatures and pHs

Temperature (°C)	pH	Kobs (hr^{-1})
60	7.5	0.0175
	6.5	0.0018
	6.0	0.00127
	5.5	0.00117
50	7.5	0.0064
	6.5	0.00058
	6.0	0.00050
	5.5	0.00039

reciprocal plot of 1/DC versus $1/[H^+]$ results in a straight line as shown in Figure 3. This agrees with the relationship (14):

$$\frac{1}{DC} = \frac{1}{PC} + \frac{Ka}{PC\,[H^+]}$$

where Ka is the dissociation constant of indomethacin and PC is partition coefficient. From the slope and the intercept, therefore, pKa and PC can be calculated and were found to be 4.53 and 8800, respectively.

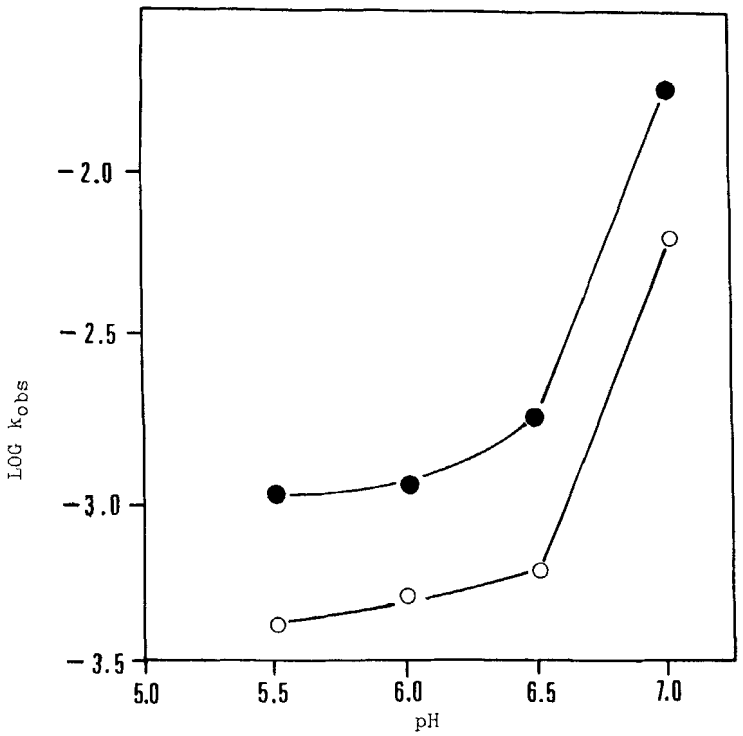

FIGURE 2

The log Kobs-pH profiles for the degradation of indomethacin in aqueous solution at different temperatures.
Keys: (-○-), 50 °C and (-●-), 60 °C.

TABLE 2

The Relationship between the pH and the Distribution Coefficient of Indomethacin in Octanol/Phosphate Buffer at 35 °C

pH	DC[a]	$(1/DC)\times10^{3}$	$(1/[H^{+}])\times10^{-5}$
6.74	52.0	19.2	57.97
6.51	82.2	12.1	32.89
6.01	250.1	4.0	11.39
5.51	960.3	1.0	3.24

[a]DC is the distribution coefficient.

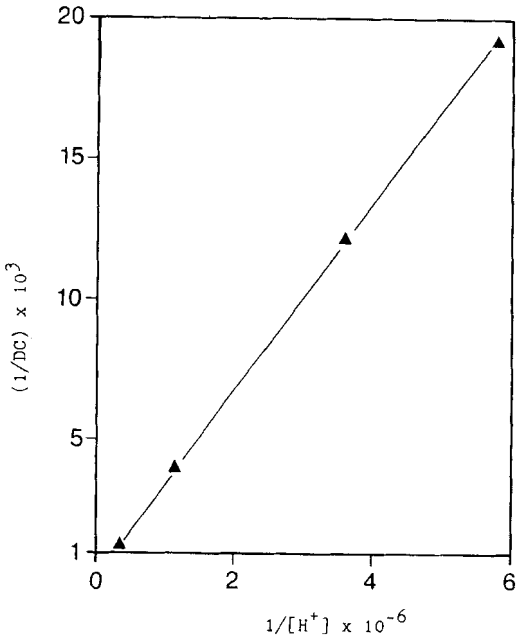

FIGURE 3

Double reciprocal plot for the determination of partition coefficient and the pKa of indomethacin.

Percutaneous Absorption Studies

Figure 4 shows the penetration profiles of indomethacin in aqueous solution at different pHs through the excised hairless mouse skin at 35 °C. The apparent skin permeability coefficient (P_{app}) was calculated from the slope according to following equation (17).

$$P_{app} = \frac{\Delta Q\%\ V\ 0.01}{\Delta t\ (3600)\ A} \qquad (3)$$

where V is the volume of the idomethacin solution on the donor

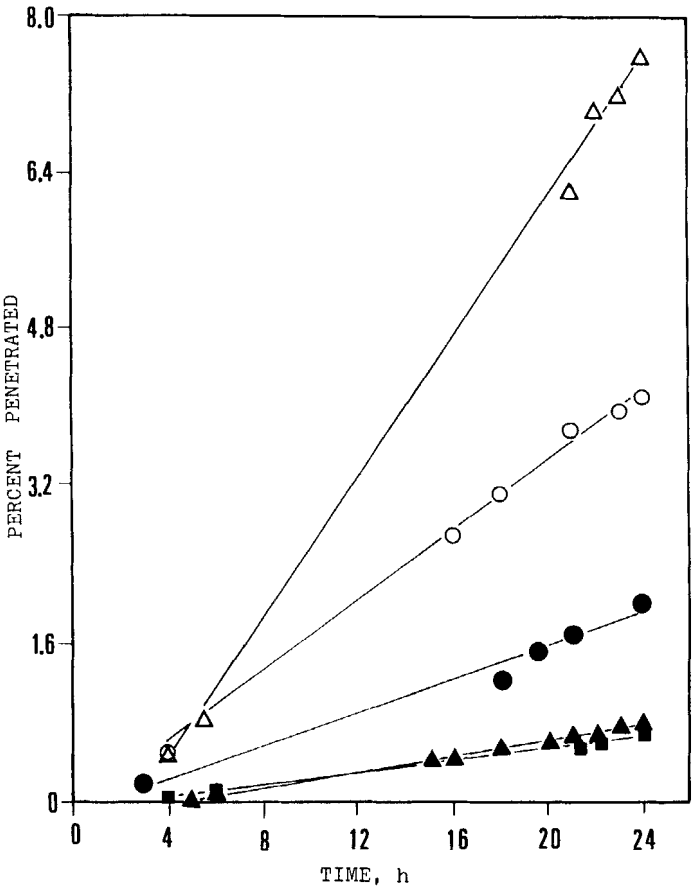

FIGURE 4

In vitro penetration of indomethacin from aqueous solutions at different pHs.
Keys: (-△-), pH 5.0; (-○-) pH 5.5; (-●-), pH 6.0; (-▲-), pH 6.5 and (-■-), pH 7.5.

side, A is the surface area (1.2 cm^2) of the orifice of the donor cell, Q represents the percentage of drug penetrated the skin from the donor cell into the receptor side. The term $Q\%/\Delta t$ is the slope, which represents the penetration rate of the drug through the skin, and 3,600 is the factor converting hours to

TABLE 3

The Apparent Mouse Skin Permeability Coefficient and Octanol/Phosphate Buffer Distribution Coefficient of Indomethacin at Different pHs and 35 °C

pH	Distribution[a] coefficient	Permeability coefficient (x 10^6, cm/sec)
5.0	2244	1.33
5.5	859	0.65
6.0	291	0.34
6.5	94	0.16
7.5	9.5	0.14

[a]Distribution coefficient is calculated according to equation 2 from calculated pKa (4.53) and partition coefficient (8800).

seconds. The calculated values were listed in Table 3. Figure 5 shows the *in vitro* absorption from 2 % indomethacin patches containing different concentrations of sodium cholate as enhancer. The absorption from the patches containing no enhancer was also included for comparison. Figure 6 shows the *in vivo* absorptions from 2 % indomethacin patches containing 6 % sodium cholate and no enhancer, respectively.

DISCUSSION

Effect of pH on Percutaneous Absorption

From Figure 2 it is seen that indomethacin is more stable under acidic conditions. The slopes of both curves between pH 6.5

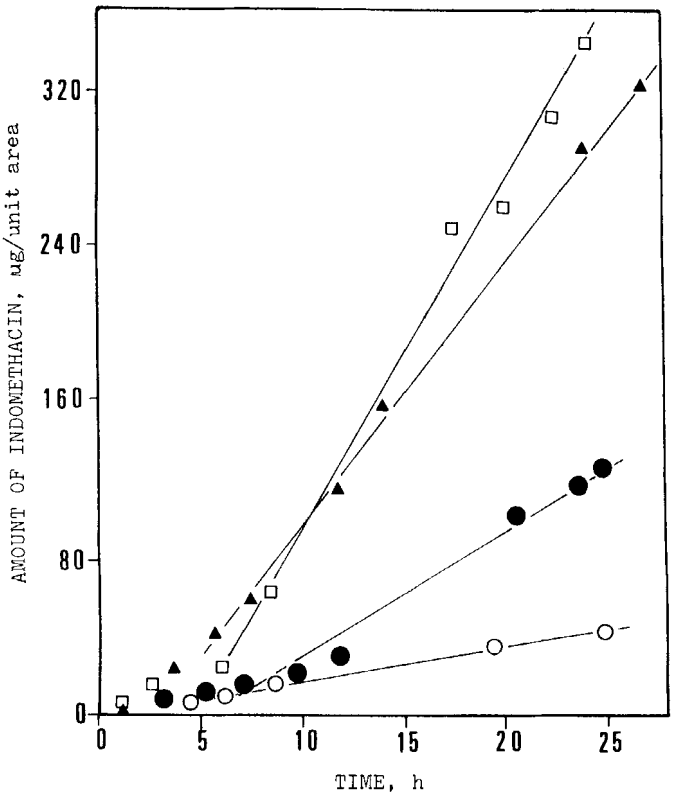

FIGURE 5

In vitro penetration from 2 % indomethacin patches containing different concentrations of sodium cholate.
Keys: (-●-), 2 % sodium cholate; (-▲-), 4 % sodium cholate; (-□-), 6 % sodium cholate; (-○-), 0 % sodium cholate.

and pH 7.0 approach to 1.0, suggesting a specific base catalysis (18, 19).

The correlation between distribution coefficient and percutanous penetration of indomethacin in aqueous solution was found to fit the following two-degree polynomial equation:

$$\log P_{app} = 0.217 (\log DC)^2 - 0.480 \log DC + 2.339$$

n=5 r=0.995 p=0.0097

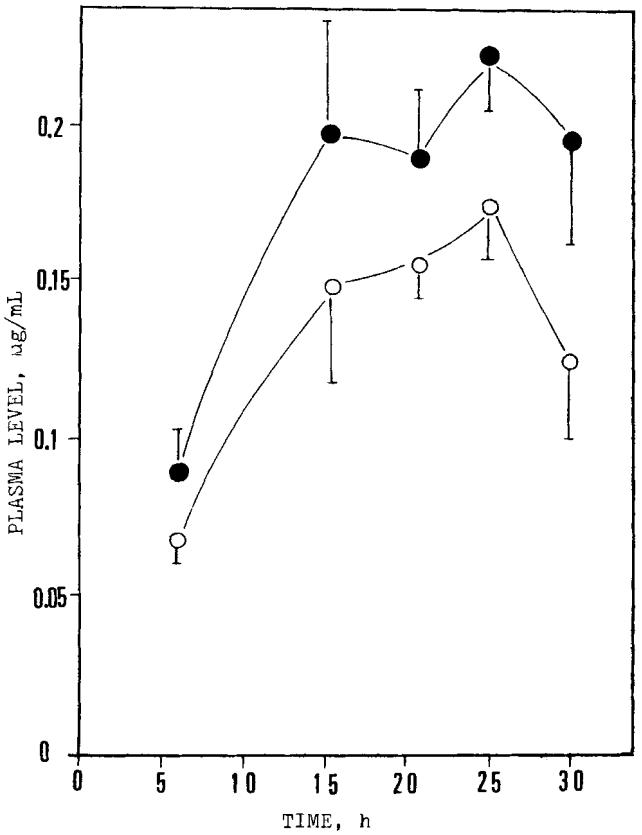

FIGURE 6

The plasma level from the application of two pieces of 2 % indomethacin patches on the back of rabbit.
Keys: (-●-), patches with 6 % sodium cholate;
(-○-), patches without enhancer.

A similar correlation had been found in the corneal penetration of eleven beta-blocking agents by Huang and Schoenwald (20).

In this study, we have found percutaneous penetration increases as the pH decreases. The stability of the drug is also improved. Since the solubility of indomethacin becomes very small

TABLE 4

In vitro Percutaneous Absorption Rate from 2 % Indomethacin Patches Containing Various Amount of Enhancers and the Total Amount of Indomethacin Penetrated During 24 Hours

Enhancers	Penetration Rate ($\mu g/hr.cm^2$)	Amount Penetrated during 24 hrs ($\mu g/cm^2$)
Without enhancer	1.89	39.5
1% Azone	1.91	44.9
2% Azone	2.05	56.7
1% Dimethyl sulfoxide	1.19	23.7
2% Dimethyl sulfoxide	1.51	33.6
3% Dimethyl sulfoxide	2.22	42.7
1% Stearyl alcohol	0.35	7.3
4% Stearyl alcohol	1.01	18.5
2% Sod. lauryl sulfate	2.32	49.9
4% Sod. lauryl sulfate	7.75	167.6
2% Sodium cholate	5.62	105.4
4% Sodium cholate	11.50	267.7
6% Sodium cholate	14.60	284.6

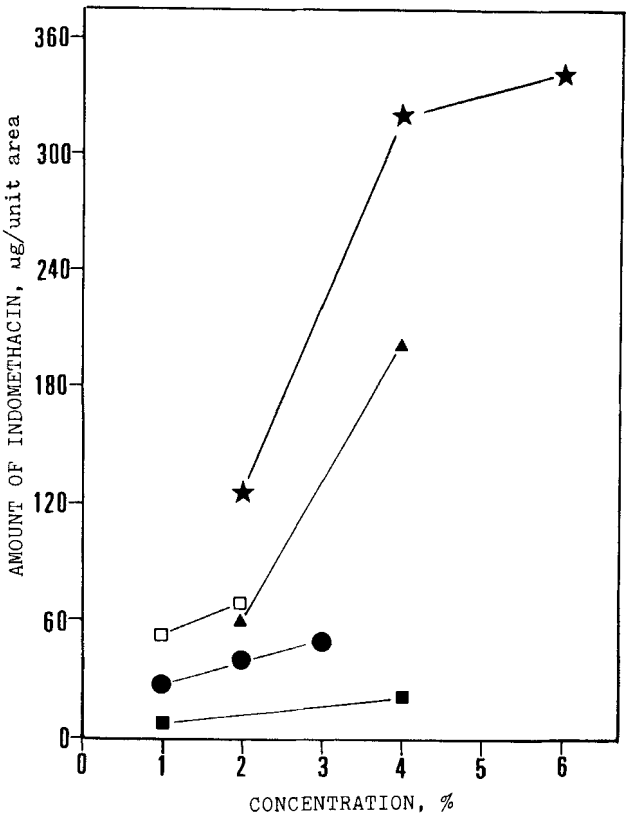

FIGURE 7

The total amount of indomethacin penetration at 24 hours from patches with different enhancers at various concentrations.
Keys: (-★-), sodium cholate, 2, 4 and 6 %;
(-▲-), sodium lauryl sulfate, 2 and 4 %;
(-□-), azone, 1 and 2 %;
(-●-), dimethyl sulfoxide, 1, 2 and 3 %;
(-■-), stearyl alcohol, 1 and 4 %.

at pH near or lower than the pKa of the drug, it is obvious that an optimal pH for the preparation of indomethacin transdermal delivery system is near 5.

Effect of Enhancers on the In Vitro Percutaneous Absorption

The percutaneous absorption rates from these patches were determined from the slope of the plot of the cumulative amount of

drug penetrated versus time. The slope was divided by the orifice area of the Franz diffusion cell (1.20 cm^2) to obtain a unit of $\mu g/hr.cm^2$. The total amount of the drug penetrated during 24 hours was also calculated from the regression line. Table 4 shows the results of these calculations. The comparison of the total amount penetrated during 24 hours from patches containing various concentrations of 5 enhancers was also shown in Figure 7. It is apparent that some chemicals did significantly improve the in vitro penetration of this drug from the device. Two surfactants, sodium lauryl sulfate and sodium cholate, showed the greatest effect. Indomthacin patches containing 4 % and 6 % sodium cholate resulted in a drug penetration about 7 times more and faster than that from the control. The dimethyl sulfoxide and azone have been reported to facilitate the percutaneous absorption of many drugs (21-23). We found that the improvement of indomethacin absorption from these two substances at the concentration employed was insignificant. Dimethyl sulfoxide usually requires a high concentration such as 80 % to achieve on enhancement of penetration. It is, however, not feasible to incorporate such high concentration of dimethyl sulfoxide in our patches.

A 3 % w/v azone emulsion was shown to enhance the permeability of 5-fluorouracil by 10-100 times in the full thickness hairless rat skin (22). In our studies, azone was incorporated into the patches and the slow release of azone from the patch, might be responsible for its ineffectiveness as an enhancer.

Stearyl alcohol is a stiffening agent and also a humectant. The amount of drug penetrated from the patch containing this compound is actually smaller compared to that from the control. More studies will be needed for an explanation.

In Vivo Study

In Figure 6 the patch with 6 % sodium cholate was shown to be better than the patch without enhancer; the AUC from initial to 30 hours was also about 1.5 times larger. However, the ratio is much less compared to the results of in vitro study. This may be partly attributed to the difference in species employed in these studies. The two patches were removed from each rabbit after treatment for one day, and the skin did not show any noticeable swelling or became reddish. Therefore, no skin irritation had occurred under the experimental conditions in this study.

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