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RESEARCH PAPER

Permeation Studies Comparing Cobra Skin with Human Skin Using Nicotine Transdermal Patches

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ABSTRACT

*Cobra skin (*Naja Naja Khaotia*) was used as a barrier for an in vitro permeation study using nicotine. Fluxes of nicotine that permeated from Nicotinell® through cobra skin (CS) taken from the head, body, and tail were 233.93 ± 16.08, 206.87 ± 19.00, and 211.26 ± 22.93 μg/cm²/hr^1/2, respectively (*$n = 6$*). This indicated no significant difference (*$p > .05$*). Abdominal human epidermis (HE), obtained from cadavers, and the CS provided identical permeation kinetics for nicotine, which can be described by* $M_t = 4M_\infty (Dt/\pi L^2)^{1/2}$*. The mean flux of nicotine formulated as an acrylic transdermal patch that permeated through HE was 137.92 ± 67.79 μg/cm²/hr^1/2 (4 specimens,* $n = 12$*), whereas that through CS was 180.13 ± 41.05 μg/cm²/hr^1/2 (4 specimens,* $n = 15$*). The ratio of the fluxes of nicotine from formulated patches having three different nicotine contents using CS and HE was 1.22 to 1, respectively, for each of the patches irrespective of nicotine content. The coefficients of variation of the nicotine permeated were 22.79% and 49.15% for CS and HE, respectively, that is, a narrower variation of results was obtained with CS. This indicated that CS could be used for nicotine permeation studies.*

Key Words: *Cobra skin; Human skin; Nicotine; Permeation; Transdermal patches.*

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INTRODUCTION

Transdermal delivery of nicotine, in the form of patches, is used as a part of the therapy for smoking cessation. Nicotine transdermal patches (NTPs) can deliver and maintain concentrations of nicotine needed in such therapy for as long as 24 hr. It also overcomes the problem of gastrointestinal discomfort caused by nicotine that has been observed with chewing gum containing nicotine and consequently improves patient compliance (1). Two types of NTPs, membrane and monolithic matrix, were studied by Ho and Chien (2). The patches using monolithic matrices were particularly interesting due to the ease of fabrication and the lack of dose dumping, which was experienced when the membrane type was employed (3). Moreover, some polymer matrices, such as polyacrylate, possess adhesive properties and can provide sustained release as well as improve product performance (4–6).

A model animal membrane used to evaluate the permeation of many drugs is shed snake skin from *Elaphe obsolata* (black rat snake) (7–9). The shed snake skin is a nonliving pure stratum corneum with no hair follicles. Similarities between the stratum corneum of the skin of black rat snakes and humans include thickness and lipid content. However, black rat snake has limited availability in Thailand. For this reason, it was decided to investigate the skin of the readily available cobra *Naja Naja Khaotia* as a model membrane. The cobra skin (CS) is easy to handle, and one snake provides enough material for a complete study.

Formulated NTPs using acrylic pressure-sensitive adhesive emulsion as the polymer matrix were evaluated for the permeation of nicotine and were compared with the permeation of nicotine from Nicotinell®.

MATERIALS AND METHODS

Materials

Acrylic pressure-sensitive adhesive emulsion and siliconized paper were a gift from Neoplast Company, Limited, Patumthani, Thailand. Polyolefin backing membrane (CoTran™ 9722) was a gift from 3M Pharmaceuticals (USA). Nicotinell (Ciba-Geigy, Basel, Switzerland) was purchased and used as supplied. Cobra skin was provided by the Thai Red Cross, Bangkok, Thailand. Methanol, high-performance liquid chromatography (HPLC grade), and (−)-Nicotine were purchased from Baker, Incorporated (Phillipsburg, NJ), and Fluka (Buchs, Switzerland), respectively. Other reagents used were analytical grade and were used as received.

Preparation of Model Membranes

CS was equally divided into 3 parts: head, body, and tail. The ventral portion was removed, and the dorsal portion was covered with sodium chloride to prevent its decomposition and was stored at 8°C. The storage times of up to 6 months did not alter the permeation of nicotine through CS. Prior to use, a piece of CS was cut, rinsed several times with distilled water until all sodium chloride was removed, and hydrated in Sörensen modified phosphate buffer, pH 7.4, for 30 min.

Thai male abdominal skin was obtained from the Department of Pathology, Faculty of Medicine, Khon Kaen University, within 24 hr of the death of people 46–50 years old. The skin selected was from cases without hair. The human epidermis (HE) was taken after the whole skin had been soaked in water at 60°C for 2 min and was stored at −20°C until use. Prior to use, the HE was hydrated by soaking in Sörensen modified phosphate buffer, pH 7.4, for 30 min.

Nicotine Transdermal Patches

Preparation

Nicotine was dispersed and evenly mixed with acrylic pressure-sensitive adhesive emulsion. The mixture was centrifuged at 3000 rpm for 10 min to remove air bubbles and then was cast on a piece of polyolefin backing membrane that had been fixed onto a clean glass plate. The thickness of the nicotine layer was controlled by a thin-layer chromatography (TLC) plate scraper. The film was dried at 50°C for 2 hr and then was covered with siliconized paper.

Characteristics

Determination of Nicotine Content

An accurately measured piece of NTP was soaked in 50 ml of Sörensen modified phosphate buffer of pH 7.4 and magnetically stirred until the maximal content of nicotine was determined. This took about 48 hr. The solution was collected and analyzed using an HPLC method. The total release of nicotine divided by the area gave the nicotine content per unit area.

Analysis of Nicotine

Reversed-phase HPLC using a C-18 column (Spherisorb® ODS-2, 5 μm, 4.6 × 250 mm) was employed. The

mobile phase was 0.05 M sodium acetate : methanol (88 : 12 v/v) containing 0.5% triethanolamine; the pH was adjusted to 4.2 with glacial acetic acid. The HPLC system used was as follows: flow rate 1 ml/min, ultraviolet (UV) detection at 259 nm, and paracetamol internal standard. The retention times of nicotine and paracetamol were approximately 5.3 and 8.4 min, respectively. Under these conditions, good linearity and reproducibility were shown over the range 1–400 μg/ml of nicotine free base.

Weight/Area Ratio of Matrix

Accurately measured portions of NTP were cut and weighed. The difference in weights between the NTP and the backing membranes when divided by the areas gave the weight/area (W/A) ratio of matrix.

Skin Permeation Studies

A 6-ml modified Franz diffusion cell (diameter 1 cm) was used. Each of the cells was stationed in a cell-mounting block having a receptor compartment that was thermostatically maintained at 37°C by jacketed water. The receptor medium, Sörensen modified phosphate buffer at pH 7.4, was stirred at 600 rpm. A piece of hydrated skin was mounted on the diffusion cell by facing the stratum corneum surface to the donor compartment and equilibrating for at least 20 min prior to use. Then, the nicotine patches (diameter 0.84 cm) were placed on the skin, which was fixed with an O-ring and fastened tight with a clamp. The amount of nicotine permeated was detected by collecting 0.4-ml samples at 1.5, 2, 4, 6, 8, 10, 12, and 24 hr. The volume of fluid withdrawn was replaced with fresh Sörensen modified phosphate buffer, pH 7.4. The concentration of nicotine in the receptor compartment was analyzed by the HPLC method as previously described.

The analysis of variance (ANOVA) test and the Student *t* test were used to determine the significant differences of permeation data.

RESULTS AND DISCUSSION

Characteristics of Nicotine Transdermal Patches

The characteristics of the NTPs studied are shown in Table 1. In appearance, all preparations were transparent, and their surfaces were smooth, indicating that nicotine was presented in the form of a solution in the film of the polymer matrix. The W/A ratios of the films loaded from different formulations demonstrated low standard deviations. This indicated good reproducibility in the production of the NTPs. The content of nicotine in NTPs, calculated as nicotine content per total weight of the film, was found to be 82–85% of the total amount added. This indicated high nicotine evaporation during the preparation, similar to that reported previously (10).

Permeation Studies Using Various Parts of Cobra Skin

The CS was divided into three parts: head, body, and tail. It was subjected to permeation studies using Nicotinell (lot 184700) as the standard nicotine patch. The permeation profiles of nicotine across various parts of CS are shown in Fig. 1. The relationship was not linear, indicating that the permeation of nicotine did not follow zero-order kinetics. However, the cumulative amount of nicotine permeated shows good linear correlation with the square root of time (r^2 higher than .99) (Fig. 2).

These results indicate that the donor system was a solution or a dissolved system; in other words, the matrix-skin system formed a single homogeneous polymeric film. The equation used to describe the transport of nicotine across CS is as follows (11):

$$M_t = 4M_\infty(Dt/\pi L^2)^{1/2},\ 0 \le M_t/M_\infty < 0.6 \qquad (1)$$

where M_t is the amount of drug released at a given time, M_∞ is the amount of drug released at infinite time, D is the diffusion coefficient of the drug in the matrix, L is the thickness of the matrix, and t is the time. An approximation of Eq. 1 could be shown by plotting the cumulative amount of drug permeated and the square root of time $t^{1/2}$ as presented by Eq. 2:

$$M_t = kt^{1/2} \qquad (2)$$

where k is the skin permeation flux.

Therefore, Eq. 2 could be used for elucidating the permeation kinetics of the nicotine permeation from NTP, the so-called NTP half-order device.

The skin permeation fluxes of nicotine released from Nicotinell using different parts (i.e., head, body, and tail) of CS given in Table 2 were not significantly different ($p > .05$). Hence, all the CS from the various body locations could be used as a barrier membrane in the evaluation of the permeation of nicotine.

Comparison of Human Epidermis and Cobra Skin Using Nicotine Transdermal Patches

The permeation profiles of nicotine from NTPs with various nicotine contents for CS and HE are presented

Table 1

Characteristics of Nicotine Transdermal Patches

Code	Weight/Area Ratio, g/cm^2 (SD $\times$ 10^3) (n = 5)	Nicotine Content (n = 3)		% of Initial Amount Added[b]
		mg/cm^2 (SD)	%w/w[a]	
A	0.0354 (0.67)	1.66 (0.11)	4.70	85.45
B	0.0342 (0.61)	2.11 (0.06)	6.17	82.27
C	0.0347 (1.10)	2.94 (0.40)	8.41	84.10
D	0.0356 (1.50)	6.03 (0.45)	17.04	85.21
E	0.0139 (0.13)	0.84 (0.01)	6.12	81.66
F	0.0573 (0.97)	3.66 (0.18)	6.39	85.15

[a] % nicotine content = (Nicotine content $\times$ 100)/(Weight/Area ratio).
[b] % of initial amount added = (% Nicotine content $\times$ 100)/% Initial nicotine content in polymer.

in Figs. 3a and 3b, respectively. The cumulative amount of nicotine permeated and the $t^{1/2}$ exhibited a similar linear relationship ($r^2 \geq .98$); this is shown in Table 3. CS provided a higher permeation flux of nicotine than HE. Figure 4 presents the linear relationship of the skin permeation flux of nicotine using NTPs having three nicotine contents across both CS and HE. The slope of this graph was 1.22 with a correlation coefficient over .99, indicating that the permeation fluxes of nicotine using CS were 1.22 times higher than those using HE. This suggested a greater permeability coefficient for CS.

Interspecimen variation of CS and HE were investigated using NTP (code B) because the permeation flux across CS was close to that of Nicotinell (data shown in Tables 2 and 3). The permeation profiles of nicotine

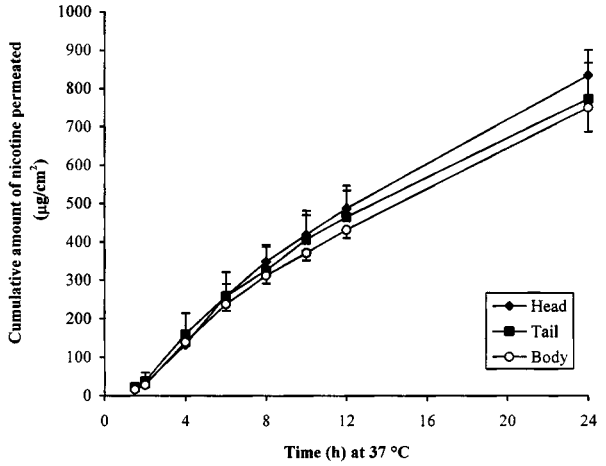

Figure 1. Comparative permeation profiles of nicotine delivered from Nicotinell across various parts of CS. Each point represents the mean ± SD of 6 determinations.

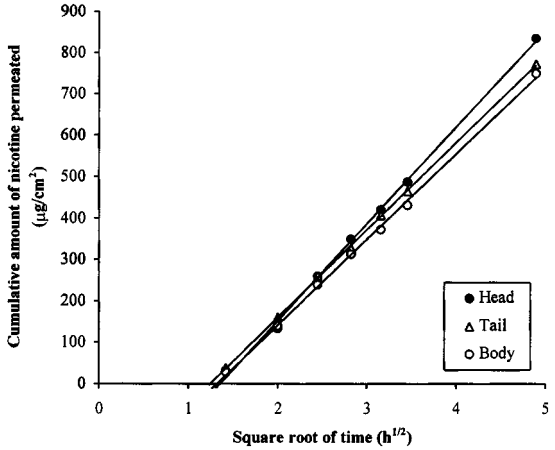

Figure 2. Relationship between the cumulative amount of nicotine permeated and $t^{1/2}$ for various parts of CS.

Table 2

Permeation Flux of Nicotine Through Various Parts of Cobra Skin Using Nicotinell

Part of Cobra Skin	Permeation Flux[a] (μg/cm^2/hr$^{1/2}$)
Head	233.93 ± 16.08 (r^2 = .9982)
Body	206.87 ± 19.00 (r^2 = .9986)
Tail	211.26 ± 22.93 (r^2 = .9996)

[a] Each value represents the mean ± SD of 6 determinations.

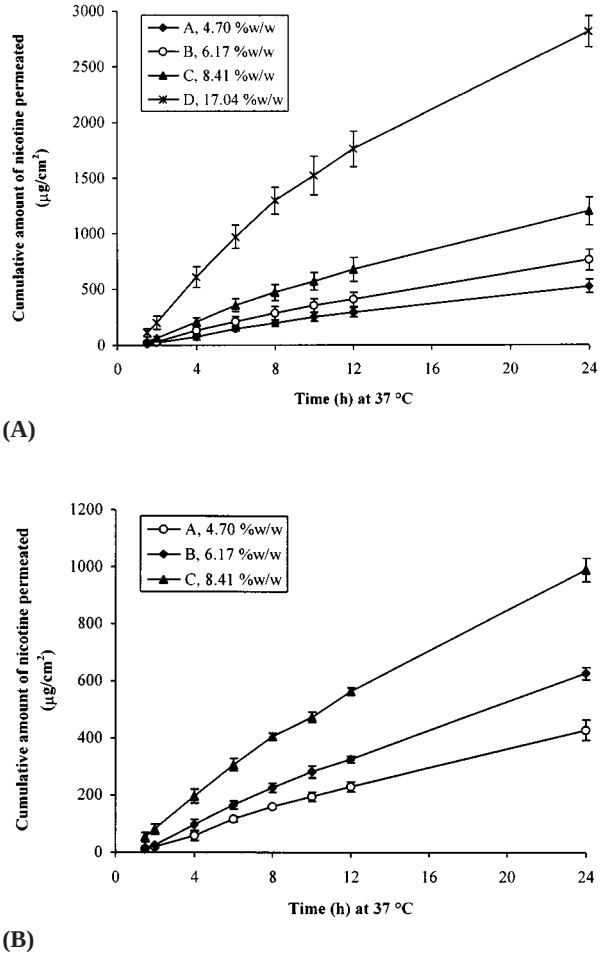

Figure 3. Permeation profiles of nicotine delivered from various nicotine contents of NTPs across (A) CS and (B) HE. Each point represents the mean ± SD of 6 determinations for CS and 3 determinations for HE.

across four specimens of CS and HE are presented in Fig. 5. It was observed that CS permeation of nicotine gave a lower variation (lower standard deviation) than HE. The mean fluxes of CS were higher than those of HE (Table 4), but were not statistically different ($p > .05$). It can be seen that the mean flux of nicotine across CS was 1.31 times higher than for HE. This value was similar to the linear relationship of the skin permeation flux of nicotine using NTPs having three nicotine contents across both CS and HE. However, using ANOVA, both types of skin varied significantly ($p < .05$) when the interspecimen permeation fluxes were evaluated. The advantage of CS observed here was that the percentage coefficient of variation (%CV) of permeation flux obtained from four specimens was lower than for HE. These results agree with

Table 3

Permeation Flux of Nicotine Across Cobra Skin and Human Epidermis from Nicotine Transdermal Patches

	Permeation Flux[a] ($\mu g/cm^2/hr^{1/2}$)	
Code	Cobra Skin	Human Epidermis
A	146.79 ± 16.58 (r^2 = .9937)	118.93 ± 9.24 (r^2 = .9898)
B	209.27 ± 26.54 (r^2 = .9921)	173.17 ± 7.25 (r^2 = .9874)
C	319.66 ± 43.48 (r^2 = .9948)	262.23 ± 12.19 (r^2 = .9924)
D	757.13 ± 58.64 (r^2 = .9995)	—
E	133.59 ± 9.98 (r^2 = .9970)	—
F	304.94 ± 49.43 (r^2 = .9874)	—

[a] Each value represents the mean ± SD of 6 determinations for CS and 3 determinations for HE.

a previous report that stratum corneum of human abdominal skin prepared by heat separation and trypsinization provided 42%CV in vitro (12); this is of a similar order to the results presented here. The human skin gave a greater interspecimen variation because of the variation of hair follicles (13) and the thickness of the epidermis (14).

These results indicate that CS should offer advantages in terms of lower %CVs. The ease of handling and ready

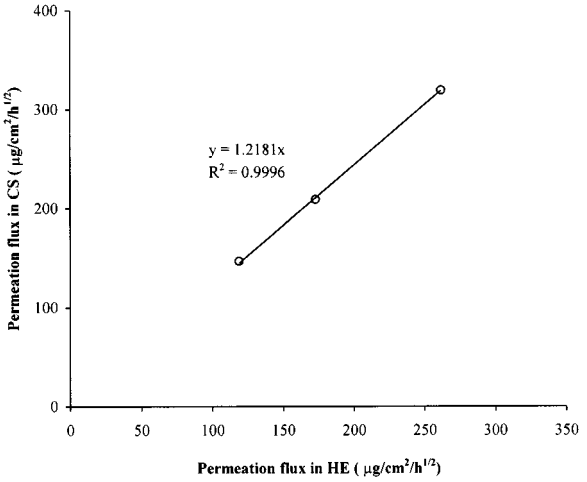

Figure 4. Relationship of permeation flux of nicotine across CS and HE using various nicotine contents of NTPs.

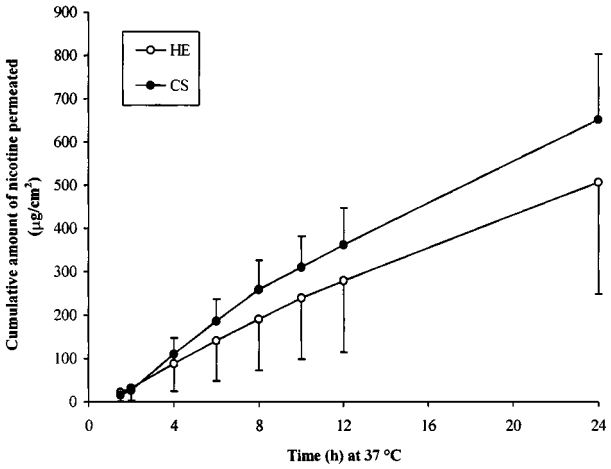

Figure 5. Comparative permeation profiles of nicotine delivered from NTPs (code B) across four specimens of CS and HE. Each point represents the mean ± SD of 15 determinations for CS and 12 determinations for HE from four specimens.

availability in Thailand are also important factors. HE, on the contrary, provided higher %CVs, indicating wide fluctuation of results. There are also greater difficulties in obtaining suitable samples.

Effect of Nicotine Content on Permeation Profiles

The nicotine contents of NTPs in codes A–D were 4.70%, 6.17%, 8.41%, and 17.04% w/w, respectively. All formulations had the W/A ratios in the range 0.0342–0.0356 g/cm^2. The permeation fluxes of nicotine through CS from NTPs of various nicotine contents are presented in Table 3. It can be seen that the permeation flux of nicotine increased when the nicotine content increased. An increase in the nicotine content in the polymer matrix provided a reduction of the relative amount of polymer as a diffusional barrier, which gave increased release of nicotine. Thus, the higher concentration gradient provided greater permeation of nicotine. Moreover, the permeation flux also increased steadily with increasing nicotine content in the polymer matrix (r^2 higher than .99).

Table 4

Interspecimen Variation of Permeation Flux of Nicotine Across Cobra Skin and Human Epidermis Using Nicotine Transdermal Patches (Code B)

	Permeation Flux[a] (μg/cm^2/hr$^{1/2}$)	
Specimen	Cobra Skin	Human Epidermis
1	209.27 ± 26.54	80.93 ± 2.62
2	151.67 ± 2.34	214.88 ± 53.92
3	131.35 ± 32.21	82.72 ± 45.97
4	199.07 ± 33.51	173.17 ± 7.25
Mean[b]	180.13	137.92
SD[b]	41.05	67.79
%CV	22.79	49.15

[a] Each value represents the mean ± SD of three determinations except specimen 1 of cobra skin (n = 6).
[b] n = 15 for CS, and n = 12 for HE.

Effect of Weight/Area Ratio of Matrix on Permeation Profiles

The W/A ratios of matrix in codes E, B, and F were 0.0139, 0.0347, and 0.0573 g/cm^2, respectively, while the nicotine contents were relatively constant. The permeation profiles presented in Fig. 6 show that higher W/A ratios of matrix result in greater amounts of nicotine permeated. However, the plot between the cumulative amount of nicotine permeated versus $t^{1/2}$ showed straight lines that followed Eq. 2. The skin permeation flux of the greater W/A ratios of matrix was higher than for lower W/A ratios of matrix (Table 3).

The effect of medicament thickness on skin permeation can be elucidated as indicated by Guy and Hadgraft (15):

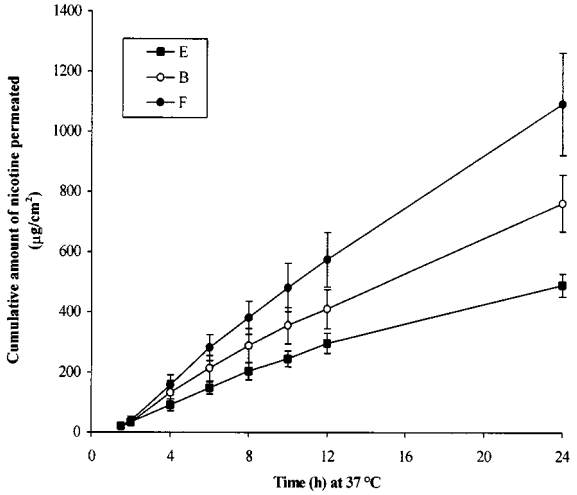

Figure 6. Permeation profiles of nicotine delivered from various W/A ratios of matrix of NTPs across CS. Each point represents the mean ± SD of 6 determinations.

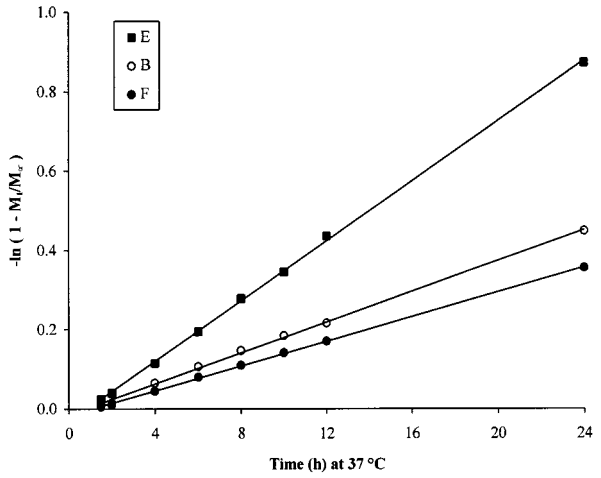

Figure 7. Relationship between $-\ln(1 - M_t/M_\alpha)$ and t obtained from various W/A ratios of matrix of NTPs.

$$-\ln(1 - M_t/M_\infty) = D_s t/KL_oL_s \quad (3)$$

in which D_s is the diffusion coefficient of the drug in the skin, K is the partition coefficient of the drug between medicament and skin, L_o is the thickness of medicament, and L_s is the thickness of the skin. This equation could be applied in the case of long-time approximation.

From this equation, the relationship between $-\ln(1 - M_t/M_\infty)$ and t is shown in Fig. 7. It was seen that the decrease of the drug in the polymer matrix followed simple first-order kinetics with a rate constant (D_s/KL_oL_s). In the greater thickness (greater W/A ratio of matrix), the remaining nicotine in the matrix at a given time was higher than at the lower W/A ratio of the matrix. However, the amount of nicotine permeated from the greater W/A ratio matrix in NTP preparations was higher than that for the lower W/A ratio of the matrix. In addition, the skin permeation flux and the W/A ratio of matrix presented a good linear relationship (r^2 higher than .99). It was observed that the skin permeation flux depended on the W/A ratio of the matrix of NTP. Hence, this parameter is very important for the permeation profiles of NTP.

CONCLUSION

The permeation profiles of nicotine patches across CS and HE had identical kinetic profiles that showed a linear relationship and a good correlation between the cumulative amount of nicotine permeated and $t^{1/2}$. The permeation fluxes of the kinetic profiles had a good correlation. Both CS and HE showed interspecimen variations. The values of permeation fluxes obtained with the CS gave lower %CVs of permeation parameters than those obtained with the HE. Thus, CS could be used as a potential membrane to evaluate nicotine permeation since it demonstrates low intra- and interspecimen variations. It also has the possibility for evaluating transdermal penetration of other active moieties with a consequent reduction of the use of human skin and synthetic membranes. This study also indicates that the crucial parameters for quality control of the production of NTPs consist of the nicotine content and the W/A ratio in the polymer matrix.

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