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RESEARCH PAPER

In Vitro Evaluation of the Release of Albuterol Sulfate from Polymer Gels: Effect of Fatty Acids on Drug Transport Across Biological Membranes

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ABSTRACT

In this investigation, the diffusion of the beta$_2$ agonist albuterol sulfate (ABS) across several membranes (cellulose, hairless mouse skin, human cadaver skin) from polymer gels was studied, and the effects of several fatty acids on drug permeation through skin were evaluated. The results were then used to predict whether transdermal delivery would be appropriate for ABS. All in vitro *release studies were carried out at 37°C using modified Franz diffusion cells. In preliminary studies, ABS release through cellulose membranes was studied from two polymeric gels, Klucel® (hydroxypropylcellulose) and Methocel® (hydroxypropylmethylcellulose). Three polymer concentrations were used for each gel (0.5%, 1.0%, and 1.5%). From these experiments, Klucel 0.5% was selected as the optimal formulation to study ABS diffusion across hairless mouse skin. Experiments were conducted to evaluate the effects of capric acid, lauric acid, and myristic acid as penetration enhancers. The results suggested that lauric acid preferentially enhanced ABS diffusion compared to the other fatty acids studied, and follow-up studies were done to evaluate the release through human cadaver skin from a donor containing 2% ABS and lauric acid in 0.5% Klucel®.*

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These experiments showed that a 2:1 (lauric acid:ABS) molar ratio gave the best ABS release rates. The release rate across human cadaver skin declined slowly over 24 hr, and an average flux over 24 hr of ~0.09 mg/hr cm^2 was measured. Using this value as a steady-state flux, extrapolations predicted that transdermal delivery can be used to maintain therapeutic ABS plasma levels (6–14 ng/mL) for extended periods. The results of this research suggest that ABS is a good candidate for transdermal drug delivery.

Key Words: *Albuterol sulfate; Drug delivery; Transdermal; Penetration enhancers; Polymer gels*

INTRODUCTION

For many years, people have applied various agents to the skin in the form of creams, lotions, ointments, and gels for the purpose of cosmetic beautification or local dermatological therapy. Over the past two decades, interest has continued to evolve on the possibility of using a transdermal route of drug delivery for systematic therapy.[1–9]

Albuterol sulfate (ABS) is a beta$_2$ adrenergic receptor agonist used as a bronchodilator or for the relief of bronchospasm in patients with reversible obstructive airway disease. It is available in a number of dosage forms for oral (e.g., tablets and syrup) and pulmonary (aerosols and nebulizer solution) delivery. However, there are several problems associated with these routes of administration for albuterol. Orally administered albuterol undergoes significant first-pass metabolism, thereby reducing the bioavailability of the formulation to as low as 10% of the original dosage. On the other hand, aerosol delivery demands special training on the part of the patients for proper use, while the nebulizer solution requires special equipment and is used either alone or in combination with saline solution. Furthermore, ABS has a half-life of 5 hr. Thus, these delivery systems must be administered several times each day to maintain therapeutic activity. Oral dosage forms must be taken three to four times daily, while the inhalation form needs to be administered every 4 to 6 hr. In consideration of these facts, it is of interest to evaluate transdermal delivery as a route of administration for ABS.

The aim of the present study was to evaluate the suitability of the transdermal route of drug delivery for ABS. The specific goals of the research are to: (1) study the release of ABS from several topical gel preparations, and its diffusion through cellulose membrane, hairless mouse skin, and human cadaver skin from the various formulations and (2) examine the effect of various fatty acids on the percutaneous absorption of ABS.

MATERIALS AND METHODS

Materials

Albuterol sulfate (USP) and *n*-octanol were purchased from Spectrum Chemical Company (Gardena, CA). Cellulose acetate membranes (Spectra Pro®-6, MW cut-off 1000) were obtained from Spectrum Laboratories (Rancho Dominguez, CA). Capric acid, lauric acid, myristic acid, and gentamicin sulfate were purchased from Sigma Chemical Company (St. Louis, MO). Methocel® (hydroxypropylmethylcellulose) was purchased from Dow Chemical Company (Midland, MI). Klucel® HF (hydroxypropylcellulose) was purchased from Aqualon Chemical Company (Wilmington, DE). All chemicals were reagent grade.

Hairless male mice (6 weeks of age) were obtained from Harlan (Indianapolis, IN). Human cadaver skin (42-year-old male donor) was acquired from the International Institute for the Advancement of Medicine (Jessup, PA).

Partition Coefficient Determination

Albuterol sulfate is very soluble in water (1 g dissolves in 4 mL of water). The partition coefficient of ABS was determined using an *n*-octanol/phosphate buffer (pH 7.4) system. The purpose of these studies was to assess the effect of fatty acids on the partitioning behavior of ABS. The drug was equilibrated in both phases for 6 hr at 37°C. Preliminary experiments determined that equilibrium was reached

within 6 hr. Studies were carried out in the absence and presence of three different fatty acids, each at three molar ratios (fatty acid:ABS). The acids were capric acid, lauric acid, and myristic acid, in molar ratios of 1:1, 2:1, and 3:1. The concentration of the drug in the aqueous phase was determined spectrophotometrically at 276 nm. Drug concentrations were calculated from a calibration curve (standard concentration range 10 μg/mL to 100 μg/mL). All calibration curves had correlation coefficients greater than 0.99. Each experiment was performed in triplicate.

Preparation of Gel Formulations

Two different gels were used to prepare formulations of ABS: Klucel® and Methocel®. Three different concentrations of gel were prepared (0.5%, 1.0%, and 1.5%). The Klucel® gels were prepared by adding the powdered polymer into the vortex of agitated water at room temperature, at a rate that was slow enough to permit the particles to separate in the water. For the Methocel® gels, the powdered polymer was mixed in 20% of the required volume of water at a temperature between 80 and 95°C (as specified by the manufacturer). After mixing to obtain a smooth paste, the remaining 80% of the water, at a temperature of approximately 20°C, was added. In both cases, the polymer–water mixture was agitated until a smooth gel was obtained, then homogenized for 40 min at room temperature. The ABS was then incorporated into the gel as an aqueous solution, in an amount that would give the desired final concentration of ABS in the gel. Where applicable, solutions of the fatty acids in ethanol 10% were added to the gels. The prepared gels were refrigerated overnight and any trapped air was removed.

Several additional formulations were prepared. In order to measure the effect of increased drug concentration in the gel on the drug permeation, a formulation was made containing 2% ABS in 0.5% Klucel®. To study the effect of penetration enhancers on drug permeation, capric, lauric, or myristic acid was included in the formulations. The concentrations of fatty acids in the formulation were 1:1, 2:1, or 3:1 (molar ratio). These ratios were selected based on previous studies conducted in the laboratory.[10]

In Vitro Release Studies

The in vitro release studies were carried out in modified Franz diffusion cells (Crown Glass, Somerville, NJ) having 15-mm diameter and 1.76-cm^2 diffusional area. Studies were performed using Spectra Pro®-6 cellulose membranes (thickness 0.006 cm), hairless mouse skin (thickness ~0.04–0.05 cm), and human cadaver skin (thickness ~0.06 cm). According to manufacturer specifications, the cellulose membranes were soaked for 24 hr in purified water to remove traces of sodium azide (a preservative).

The receiver compartment (capacity 12.3 mL) was filled with isotonic phosphate buffer solution (PBS) (pH 7.4). The receptor solution was mixed with a magnetic stirrer. The temperature of the receiver compartment was maintained at 37 ± 0.5°C by means of a circulating water bath. An accurately measured volume of test formulation was placed in the donor compartment and the compartment sealed with Parafilm. Samples (0.3 mL) were collected from the receiver compartment using a microsyringe and replaced immediately with an equal volume of isotonic phosphate buffer solution.

Samples were transferred to a 10-mL test tube, diluted with PBS up to 3 mL, and stored frozen prior to analysis. Samples were analyzed spectrophotometrically at 276 nm for ABS content. Preliminary studies found no interference from fatty acids present in the sample. The concentration in each sample was determined by using a previously constructed standard curve. These concentrations were corrected (for dilution) in order to determine the actual cumulative amount of ABS released. All experiments were performed in triplicate.

Male hairless mice (approximately 6 weeks of age) were used as skin donors for in vitro experiments. Animals were sacrificed by inhalation using an approved protocol. The abdominal skin was excised from the animal using scissors and a scalpel, the adhering visceral debris was carefully removed from the inner surface, and the excised skin was thoroughly washed with the isotonic buffer solutions. The skin was positioned between the receiver and donor cells, with the stratum corneum of the skin facing the donor compartment, and the two cells were secured tightly by a pinch clip. Excess skin was trimmed using surgical scissors. Gentamicin sulfate solution (5 mg/mL) was added to the receiver compartment in a 1:50 ratio to

prevent microbial contamination during the experiment. Cadaver skin (full thickness) was rapidly thawed and washed in distilled water to remove glycol, which was the storage solution. Release studies were performed as described above for hairless mouse skin.

In cellulose- gel studies, the ABS concentration in the donor was 1%. In the hairless mouse skin and human cadaver skin studies, the initial donor concentration of ABS was 2%.

Data Analysis

Penetration profiles (plots of cumulative mass released as a function of time) were constructed. The average flux (J_{ave}) was calculated from the penetration profile using the equation:

$$J_{ave} = \frac{1}{A}\frac{dM}{dt}\bigg|_{ave} \quad (1)$$

Here $dM/dt|_{ave}$ is the slope of a straight line obtained by a linear regression of the M vs. t profile over the experimental timeframe, and A is the diffusional area. The permeability coefficient P was calculated from the following equation:

$$P = \frac{J_{ave}}{(C_d - C_r)_{ave}} \quad (2)$$

where C_d and C_r are the ABS concentrations in the donor and receiver, respectively, and $(C_d - C_r)_{ave}$ is the average concentration difference during the experiment, given by:

$$(C_d - C_r)_{ave} = C_{d,ave} - C_{r,ave} = C_{d,0} - \frac{M}{2V_d} - \frac{M}{2V_r}$$

where V_d and V_r represent the volume of the donor and receiver compartment, respectively.

RESULTS AND DISCUSSION

The apparent partition coefficients of ABS in the presence of various fatty acids were determined in an *n*-octanol/phosphate buffer (pH 7.4) system, and are listed in Table 1. With increasing acid:ABS ratios, it was found that the octanol/buffer partition coefficients for the ABS increased for capric acid (C_{10}) and lauric acid (C_{12}), but decreased for myristic acid (C_{14}). The partitioning of ABS alone (in the absence of fatty acid) was negligible.

The diffusion characteristics of 1% ABS gelled with two different polymeric gelling agents (Klucel® and Methocel®) were studied using cellulose membranes. These studies provided a preliminary screen for predicting drug permeation across other membranes (hairless mouse skin, human cadaver skin). Analysis of variance (ANOVA) for Klucel® and Methocel® (95% confidence interval, $P < 0.05$) indicated differences between the release profiles. The effects of varying the polymer concentration on the permeation parameters are shown in Table 2. The results are also displayed graphically in Fig. 1, which shows a plot of ABS release through cellulose as a function of time. The release profile is typical of what is seen for the release from gel through highly permeable membranes. At very early times, the release is limited by diffusion through the cellulose, after which time the release from the gel becomes

Table 1

Effect of Fatty Acids on the Apparent Octanol/Water Partition Coefficient (K_p) of Albuterol Sulfate[a]

Capric Acid (CA)		Lauric Acid (LA)		Myristic Acid (MA)	
Molar Ratio (CA:ABS)	$K_p \times 100$	Molar Ratio (LA:ABS)	$K_p \times 100$	Molar Ratio (MA:ABS)	$K_p \times 100$
1:1	3.54	1:1	7.02	1:1	9.70
2:1	8.17	2:1	11.8	2:1	9.02
3:1	10.8	3:1	14.1	3:1	5.76

[a]K_p was determined at 37°C in an *n*-octanol/phosphate buffer system (pH 7.4). Partitioning of ABS alone (no fatty acid) was negligible.

Table 2

Effect of Varying Gel (Klucel® and Methocel®) Concentrations on the Diffusion of ABS Through Cellulose Membrane

Polymer	Conc. (%)	J_{ave} (mg/cm^2 hr)[a]	$P\times10^2$ (cm/hr)[b]
Klucel®	0.5	0.488 (0.002)	8.66 (0.04)
	1.0	0.439 (0.004)	7.21 (0.06)
	1.5	0.366 (0.002)	5.39 (0.03)
Methocel®	0.5	0.422 (0.001)	6.81 (0.02)
	1.0	0.344 (0.001)	4.94 (0.01)
	1.5	0.290 (0.004)	3.89 (0.05)

[a] J_{ave} was determined using Eq. (1).
[b] P was determined using Eq. (2).
Standard deviations are in parentheses.

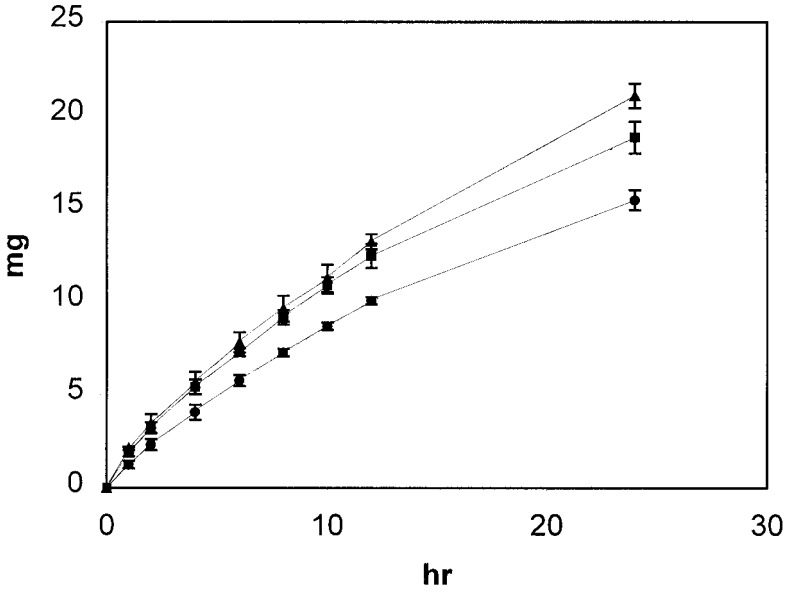

Figure 1. Albuterol sulfate release from Klucel® through cellulose membranes. The donor gels consisted of 0.5%, 1.0%, and 1.5% Klucel®, initially loaded with 1% ABS. The highest release rate was from the 0.5% gel and the lowest was from the 1.5% gel formulation. The error bars are small but still shown.

rate-limiting. This is supported by plots of the amount released as a function of the square root of time (not shown), which became linear after the first hour ($R^2 > 0.99$ for all three concentrations). From the data in Table 2, it is apparent that the release rate and associated physicochemical parameters of ABS decreased with increasing polymer concentration for both Klucel® and Methocel®. There are several possible mechanisms to explain these findings. According to the first mechanism, the polymer resists diffusion of drug molecules through the polymer chain network. At higher concentrations of polymer in the gel, the polymer chains become more crowded and more strongly inhibit drug diffusion through the gel.[10] In addition, there may be an increase in tortuosity due to the polymers

acting as microscopic barriers to diffusing drug molecules. Another possible mechanism is that the polymer chains are adsorbed on the membrane surface, forming a thin polymer layer on the membrane that partially obstructed drug release. Therefore, when the polymer concentration in the gel is increased, the amount of ABS released is decreased.

The effect of increasing the initial ABS concentration in the gel containing 0.5% Klucel® was also evaluated. It was found that when the initial donor concentration of ABS was doubled (to 2%), the flux was also doubled, in accordance with the diffusion theories.

Effect of Fatty Acids on ABS Diffusion Through Hairless Mouse Skin

Preliminary studies similar to those described, but using hairless mouse skin instead of cellulose membranes, showed that the diffusion of ABS through excised mouse skin was minimal. Therefore, follow-up studies were conducted in an effort to improve ABS permeation using fatty acids. In these studies, in vitro penetration of ABS (2%) from gel formulations (0.5% Klucel®) containing either capric acid, lauric acid, or myristic acid was determined. Three molar ratios (fatty acid:ABS) were studied (1:1, 2:1, and 3:1), and the results are provided in Table 3. It was found that, with increasing molar ratio (acid:ABS), the ABS flux increased in the presence of capric acid (C_{10}) and lauric acid (C_{12}), but decreased in the presence of myristic acid (C_{14}). It was also found that, at each molar ratio, the ABS flux was higher from formulations containing lauric acid. These results can be explained as follows.

At low concentrations, the fatty acid might interact with one or more components of the stratum corneum (e.g., free fatty acids, lipids, proteins), thereby reducing the direct interaction of these components with ABS and resulting in an increased ABS diffusion. This effect would be expected to become greater with increasing fatty acid concentration. Previously, Plakogiannis and co-workers[10] observed that increasing molar concentrations of lauric acid enhanced the membrane penetration of metaproterenol sulfate. If the fatty acid is sufficiently lipophilic, however, its presence in the skin may reduce the ability of the ABS to partition into the skin, resulting in a lower permeability. Presumably, a balance must be achieved between the amount of fatty acid present in the skin, and the effect of the fatty acid on the ability of ABS to enter the skin.

Several hypotheses have been proposed to explain the effects of increased fatty acid carbon length (C_{10}, C_{12}, and C_{14}) on skin penetration. Florence et al.[11] suggested that short-chain fatty acids have insufficient lipophilicity for skin penetration. Long-chain fatty acids (i.e., C_{12} and C_{14}) have a greater affinity for lipids in the stratum corneum than shorter chain fatty acids. Accordingly,

Table 3

Effect of Varying Molar Ratio of Three Fatty Acids on ABS Penetration Through Hairless Mouse Skin

Fatty Acid (FA)	Molar Ratio (FA:ABS)	J_{ave} (mg/cm^2 hr)[a]	$P\times10^3$ (cm/hr)[b]
Capric acid	1:1	0.025 (0.002)	1.25 (0.11)
	2:1	0.096 (0.001)	4.99 (0.06)
	3:1	0.118 (0.001)	6.19 (0.13)
Lauric acid	1:1	0.171 (0.002)	9.0 (1.1)
	2:1	0.205 (0.002)	11.0 (0.1)
	3:1	0.225 (0.003)	12.2 (0.2)
Myristic acid	1:1	0.160 (0.002)	8.38 (0.12)
	2:1	0.139 (0.002)	7.26 (0.11)
	3:1	0.074 (0.002)	3.88 (0.11)

[a] J_{ave} was determined using Eq. (1).
[b] P was determined using Eq. (2).
Standard deviations are in parentheses.

increasing the carbon chain length would increase the ability of a fatty acid to enter the skin lipids, which could result in a greater enhancement of ABS penetration through mouse skin. However, the greater lipophilicity associated with longer chain fatty acids would also reduce the ability of the ABS to partition into the skin, resulting in reduced permeation. Thus, the ability of the fatty acids to enter the skin would be a function of the carbon chain length, while the ability of the ABS to partition into the skin/fatty acid structure would be a function of the carbon chain length and the amount of fatty acid present in the skin.

This is consistent with the observations that, with increasing acid:ABS ratios, the capric acid and lauric acid systems showed increasing octanol/buffer partition coefficients and increased penetration rates, while the myristic acid systems showed decreasing octanol/buffer partition coefficients and decreasing penetration rates.

A possible explanation for why the lauric acid (C_{12}) hydrophobic group may have the greatest effect on membrane penetration may be because of an optimal balance achieved between partition coefficient and monomer concentration.[12] Dominguez et al.[13] suggested that surfactants do not adopt a linear structure in the skin but rather a coiled structure. The molecular size of the surfactants forming these coils was calculated to be smallest when the hydrophobic chain was 12 carbons in length, thereby favoring membrane penetration.

Alternatively, Florence et al.[11] suggested that lipids of similar structure tend to pack tightly together, but mixtures of long- and short-chain lipids form loosely organized structures. In the stratum corneum, major lipids are triglycerides, free fatty acids, ceramides, and cholesterol. These materials possess highly hydrophobic groups containing 16 or more carbon atoms. It is possible that the addition of shorter fatty acid chains disrupts the crystalline lipid packing, resulting in a more fluid and permeable membrane.

ABS Diffusion Through Human Cadaver Skin

Hairless mouse skin experiments revealed that lauric acid preferentially enhanced ABS diffusion compared to the other fatty acids studied. Based upon these findings, percutaneous penetration of ABS (2%) through human cadaver skin was studied using a 0.5% Klucel® gel formulation containing lauric acid at two different molar ratios (2:1 and 3:1). The results are shown in Fig. 2. Estimates for human cadaver skin permeation parameters are presented in Table 4. Analogous to findings from the hairless mouse skin studies, an explanation for these results is that at lower concentrations (2:1 molar ratio), the fatty acid might interact with some components of the stratum corneum (e.g., free fatty acids, lipids, proteins), thus preventing direct interaction between ABS and skin.

Table 4

Effect of Varying Molar Ratio of Lauric Acid on ABS Penetration Through Human Cadaver Skin

Molar Ratio (LA:ABS)	J_{ave} (mg/cm^2 hr)[a]	$P\times10^3$ (cm/hr)[b]
2:1	0.089 (0.003)	4.6 (0.2)
3:1	0.050 (0.004)	2.5 (0.2)

[a] J_{ave} was determined using Eq. (1).
[b] P was determined using Eq. (2).
Standard deviations are in parentheses.

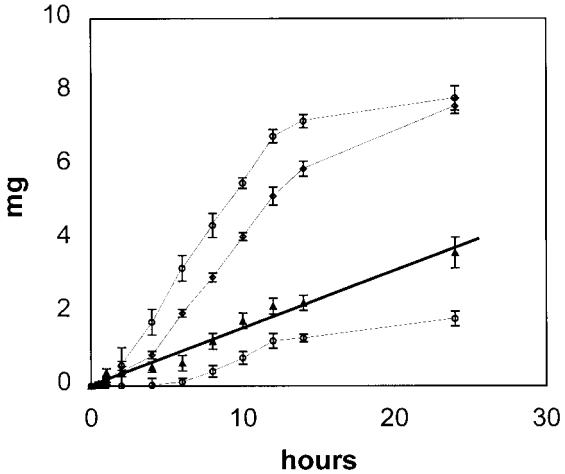

Figure 2. Albuterol sulfate release from 0.5% Klucel® and lauric acid through hairless mouse and human cadaver skin. The donor gel consisted of 0.5% Klucel® and lauric acid in 2:1 and 3:1 ratios (acid:ABS), initially loaded with 2% ABS. The data is shown in the following order (from highest to lowest amounts released): hairless mouse skin (3:1); hairless mouse skin (2:1); human cadaver skin (2:1); human cadaver skin (3:1). The solid line shown was drawn from a linear regression of the release data from the "best" human cadaver skin system (2:1 ratio).

Human skin is more complex than hairless mouse skin and, accordingly, human skin is more resistant to drug penetration than mouse skin. The stratum corneum is composed of triglycerides, free fatty acids, ceramides, and cholesterol, with hydrophobic groups of 16 or more carbon atoms. It is possible that the formulations containing higher concentrations (3:1) of fatty acid promote complexation between fatty acid and skin through hydrophobic interactions. The result of this interaction is lower permeation and diffusion of ABS through human cadaver skin. Plakogiannis and co-workers[10] studied the effect of lauric acid (2:1 and 3:1) on the percutaneous absorption of metaproterenol sulfate through hairless mouse skin. They reported that formulations containing lauric acid at a 2:1 molar ratio provided higher fluxes than formulations containing a 3:1 ratio, which is consistent with the present study.

Extrapolation of In Vitro Data to In Vivo Predictions

In a previous investigation, the pharmacokinetics of ABS were studied after intravenous and oral dosing to human volunteers.[14] Following oral administration (4 mg tablet every 8 hr), an average steady-state plasma level of 6.9 ± 1.5 ng/mL was observed. Peak ABS plasma levels were obtained between 1 and 4 hr after administration, and ranged from 10.0 to 16.9 ng/mL. The following pharmacokinetic parameter estimates were reported: half-life $t_{1/2} = 3.9 \pm 0.83$ hr, volume of distribution $V_d = 156 \pm 38$ L, clearance $CL = 28.7 \pm 7.36$ L/hr, and first-order elimination rate constant $k = 0.18 \pm 0.037\ hr^{-1}$.

Based upon the results of the present study, efforts were made to predict steady-state plasma levels following transdermal administration of ABS. If drug enters the body at a constant rate, the steady-state concentration C_{SS} can be calculated using the equation:

$$C_{ss} = \frac{AJ_{ss}}{CL}$$

where J_{ss} is the steady-state flux and A is the diffusional area. From in vitro studies with human cadaver skin using a 2:1 lauric acid formulation, the ABS steady-state flux rate was $J_{ss} = 0.152\ mg/cm^2\ hr$. Assuming this formulation is applied to human skin with an area $A = 5\ cm^2$ (approximately the area of a circular patch with a diameter of one inch), and using the pharmacokinetics parameters given above, the predicted steady-state level is approximately 15 ng/mL. This estimate falls above the reported therapeutic range of ABS plasma concentrations of ~3–14 ng/mL.[15] Based upon these predictions, therefore, it appears that suitable plasma levels of ABS can be attained through transdermal delivery.

CONCLUSIONS

The release of the bronchodilator albuterol sulfate was investigated through a variety of membranes, and its potential for use as a sustained-release transdermal formulation was evaluated. Using cellulose membranes and a variety of concentrations of Klucel® and Methocel® donor systems, the system was selected that released the drug the fastest. This system was then tested on hairless mouse skin using a variety of fatty acids as penetration enhancers. A final formulation of 0.5% Klucel® and lauric acid (2:1 lauric acid:ABS molar ratio) was selected for further study using human cadaver skin. Using a 2% initial ABS donor concentration, the release through human cadaver skin was characterized, and the results used to predict steady-state ABS plasma levels using pharmacokinetics data for ABS obtained from the literature. These calculations showed that it should be possible to obtain plasma levels two to three times higher than therapeutic levels using reasonably sized transdermal patches. It is concluded that ABS, given its pharmacokinetics data and permeation properties in human skin, would likely be an excellent choice for a sustained-release, transdermal dosage form.

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