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Permeation study of five formulations of alpha-tocopherol acetate through human cadaver skin

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Synopsis

Alpha-tocopherol (AT) is the vitamin E homologue with the highest *in vivo* biological activity. AT protects against the carcinogenic and mutagenic activity of ionizing radiation and chemical agents, and possibly against UV-induced cutaneous damage. For stability consideration, alpha-tocopherol is usually used as its prodrug ester, alpha-tocopherol acetate (ATA), which once absorbed into the skin is hydrolyzed to alpha-tocopherol, the active form. The objective of this research was to characterize *in vitro* the permeation properties of ATA from various solutions and gel formulations. Permeation studies were conducted using modified Franz diffusion cells and human cadaver skin as the membrane. Specifically, 5% (w/w) alpha-tocopherol acetate was formulated in the following vehicles: ethanol, isopropyl myristate, light mineral oil, 1% Klucel® gel in ethanol, and 3% Klucel® gel in ethanol (w/w). The receiver temperature was 37°C. Samples from the receiver were collected at 2, 4, 6, 8, 12, 24, 30, 36, and 48 hours and analyzed by HPLC for concentrations of alpha-tocopherol acetate and alpha-tocopherol. The permeabilities of ATA through human cadaver skin were 1.0×10^{-4}, 1.1×10^{-2}, 1.4×10^{-4}, 2.1×10^{-4}, and 4.7×10^{-4} cm/h for the ethanol solution, isopropyl myristate solution, light mineral oil solution, 1% Klucel® gel, and 3% Klucel® gel, respectively. The results show that the formulation had relatively minor effects on the permeability coefficients of ATA through cadaver skin in all cases except for the isopropyl myristate solution.

INTRODUCTION

Alpha-tocopherol is the major lipophilic antioxidant in many biological systems (1). The main antioxidant function of AT is to prevent lipid peroxidation at the cell membrane site and therefore to promote the preservation of the structural integrity of the membrane. AT is present in high concentration at the lower levels of the stratum corneum (2,3) where it represents the first line of defense of the skin from the oxidative stress of

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sunlight and pollutants. Indeed, formation of free radicals and subsequent lipid peroxidation is considered to be the major mechanism of UV irradiation-induced cutaneous damage (4). Exposure to UV light depletes the stratum corneum of AT, and regeneration of AT may be difficult in these conditions. Topical application of AT to the skin has been reported to protect animal skin from UV-induced damage (5) either by direct protection from free radicals or by indirect protection by means of increased epidermal thickness (6). In addition, topical application of AT may prevent mutations in critical genes (7) and effectively reduce cancer formation and immunosuppression induced by UV irradiation (8). Topical application of AT was far more effective at preventing the increase in lipid peroxidation than dietary supplementation, probably because of the higher tissue level attained (9). Other significant local actions of AT are improvement of skin microcirculation, inhibition of inflammation, promotion of hair growth, treatment of alopecia and of various skin diseases (e.g., axillar bromidrosis, chilblains, acne vulgaris, mycosis in the nail) (10,11). In addition, AT is widely used in skin care products mainly as a natural moisturizer to relieve dry skin, and as an aid in the concealment of wrinkles and facial lines.

Unfortunately, AT has a very short shelf life in topical formulations because it is sensitive to atmospheric oxygen, and it is therefore formulated as its prodrug ester, alpha-tocopherol acetate (ATA). ATA is biologically inactive because it lacks the free phenolic OH group. However, ATA is believed to hydrolyze to the active form, AT, in the skin. The skin is capable of many of the same types of metabolic processes that are present in the liver and other organs (12), but the overall metabolizing capacity of the skin is less than that of the liver by nearly two orders of magnitude. Therefore, the actual efficacy of ATA-containing products is still uncertain. The metabolic capability of the skin may differ among species. For instance, in rat skin, van Henegouwen *et al.* (13) found that after a period of five hours following a single application of ATA, the amount of AT in skin does not significantly differ from the amount already present in the skin. In pig skin, Rangarajan and Zatz (14) found that AT appeared as early as two hours after application, with the extent of metabolism reaching a peak at 6–12 hours after application. No metabolism was detected in the stratum corneum, but it was detected in the viable skin (13,15). They also demonstrated that the topical delivery and metabolism of ATA were dependent on formulation. Baschong *et al.* (15) did an *ex vivo* study in viable human skin. Their study confirms that also in humans bioconversion of ATA to AT is localized exclusively in the viable skin. Hydrolysis was absent on the skin surface as well as in the horny layer. Distribution of ATA in skin was dependent on the formulation.

The *in vitro* study of ATA permeability is particularly challenging because ATA is very poorly soluble in water, and water is usually the major component of the receiver compartment of *in vitro* testing cells. The objectives of this study were to evaluate the permeation through human cadaver skin of ATA *in vitro* from various topical formulations. The formulations tested were kept simple and ranged from solutions of increasing viscosity (ethanol, isopropyl myristate, and mineral oil) to gel formulations. A recently reported mathematical approach (16) for the determination of membrane permeability was used. The method has the advantage that accurate determinations of membrane permeabilities can be done using a common experimental technique and can be applied to systems in which the donor compartment is unstirred.

THEORY

Bellantone *et al.* (16) described in detail the mathematical model used to estimate the permeability coefficients in this study. The model corresponds to the experimental setup used in this study (modified Franz diffusion cells), in which the drug leaves an unstirred donor, crosses through a membrane of thickness h and cross-area A, and accumulates in a stirred receiver for which sink conditions are maintained. Initially, the drug concentration C_0 in the donor is uniform, while the membrane and receiver are void of drug. Fick's laws were used to give equations for the rate of accumulation of the drug in the receiver. (Table I contains a description of the symbols and abbreviations used in this paper.)

OBTAINING THE PERMEABILITY OF A RATE-LIMITING MEMBRANE

Here, the transport across the membrane is the rate-controlling step. The general equation for the cumulative amount of drug released into the receiver M at time t is given as an infinite series. However, if the times used for the data points are not too large (see below), the equation can be simplified to give

$$M = \alpha\left[\sqrt{t}\exp\left(-\frac{\beta^2}{t}\right) - \beta\sqrt{\pi}\,erfc\left(\frac{\beta}{\sqrt{t}}\right)\right] \qquad (1)$$

Table I
Definitions of Symbols and Abbreviations Used in This Paper

A	Area
AT	Alpha-tocopherol
ATA	Alpha-tocopherol acetate
C_0	Initial drug concentration
C_d	Donor drug concentration
C_m	Membrane drug concentration
D	Diffusion coefficient
D_d	Diffusion coefficient of donor
D_m	Diffusion coefficient of membrane
g	Acceleration due to gravity
h	Thickness of membrane
HPLC	High-performance liquid chromatography
K	Membrane/donor partition coefficient
L	Distance
M	Amount release
N	Avogadro's number
P_m	Membrane permeability
r	Radius of the spherical particle
R	Molar gas constant
t	Time
T	Absolute temperature (K)
UV	Ultraviolet
v	Velocity
η	Viscosity of solvent
ρ_B	Density of the spherical particle
ρ_l	Density of solvent

where

$$\alpha = \frac{4\,AKC_0\sqrt{D_d D_m}}{\sqrt{\pi}\left(\sqrt{D_d} + K\sqrt{D_m}\right)}$$

and

$$\beta^2 = \frac{h^2}{4D_m}$$

and $erfc\ u = 2/\sqrt{\pi}\int_u^\infty \exp(-w^2)dw$ is the complementary error function. Here, D_m denotes the permeability of the drug through the membrane, D_d denotes the diffusion coefficient of the drug in the donor medium, and K represents the membrane/donor partition coefficient. The parameter α has units of mass per square root of time, and β^2 has units of time. The specific requirements for the simplification of equation 1 are that the times at which the samples are taken satisfy $t < 2\text{–}3\beta^2$, and less than ~1/3 of the drug initially in the donor has left the donor (16).

The parameters α and β can be determined from the experimental release data by performing a nonlinear regression using equation 1. Detailed information on the method used to find good initial estimates of the parameters α and β is reported by Bellantone *et al.* (16). The permeability P_m of the membrane can be estimated as (16):

$$P_m = \frac{KD_m}{h} = \frac{\alpha\sqrt{\pi D_d}}{2\beta\left(4AC_0\sqrt{D_d} - \alpha\sqrt{\pi}\right)} \tag{2}$$

In this study, A = 1.76 cm^2, C_0 = 50,000 µg/ml, β ranged from 3–5 hr$^{1/2}$, and D_d > 0.01 cm^2/hr in all cases. For the ATA/isopropyl myristate formulations, α ~ 10,000, and equation 2 was used to calculate P_m. For formulations other than the ATA/isopropyl myristate, α ranged from 100 to 500 µg/hr$^{1/2}$, and less than 2% error is introduced by simplifying equation 2 as

$$P_m = \frac{\alpha\sqrt{\pi}}{8\beta AC_0} \tag{3}$$

The ATA diffusion coefficients in the various donors were estimated by two methods. In the first method, cellulose permeation data was used to obtain the diffusion coefficient in the donor, and was employed when it was possible to assay the drug in the receiver. The second method used the relative viscosities of the media to estimate the diffusion coefficient and was employed in cases where permeation data was not easily evaluated. The methods are described below.

Obtaining D_d using cellulose permeation data. Here, cellulose membranes are used in release experiments because they are thin and highly permeable. Thus, a pseudo-steady state develops in the membrane quickly, and the release of drug is primarily controlled by the donor region behavior. If the donor and receiver media are the same (to avoid solvent drag effects in the membrane), this model can be used to obtain information about the diffusion coefficient in the donor D_d. The cumulative amount of drug released is given by (17):

$$M = \kappa\left[\exp(\lambda^2 t)erfc\left(\lambda\sqrt{t}\right) + \frac{2\lambda}{\sqrt{\pi}}\sqrt{t} - 1\right] \quad (4)$$

where

$$\kappa = \frac{AD_dC_0}{P_m} \quad \text{and} \quad \lambda^2 = \frac{P_m^2}{D_d}$$

Here, κ has units of mass, and λ^2 has units of reciprocal time. Values for κ and λ can be obtained from a nonlinear regression using equation 3, allowing D_d to be calculated as

$$D_d = \left(\frac{\kappa\lambda}{AC_0}\right)^2 \quad (5)$$

In some cases, when D_d is large enough, the M-vs-t plot is linear over the entire course of the experiment, and dM/dt can be taken as nearly constant during that time interval. (This often happens when the donor is a liquid.) In this case, the method loses accuracy in calculating the actual values for D_d. However, good estimates of the lowest value of D_d that can account for the linearity can be obtained. However, D_d is evaluated only to verify that equation 3 is valid, and this information is sufficient for use in this study. The estimation is done as follows: From equation 4, the release rate is given by

$$\frac{dM}{dt} = \kappa \exp(\lambda^2 t)erfc\left(\lambda\sqrt{t}\right)$$

Using the approximation $dM/dt = AP_mC_0$, it can numerically be shown that dM/dt changes by less than 5% when $\lambda t < 0.05$ and less than 10% when $\lambda t < 0.1$. For the 10% condition, which is the less restrictive one, this leads to

$$P_m^2 t < 0.1D_d \quad (6)$$

during the experiment. In practice, P_m is estimated from the dM/dt data and t is taken as the time of the last data point in the experiment (four hours in this study), leading to $D_d > 40P_m^2$.

Estimating D_d from viscosity measurements. In some cases, using the liquid medium (i.e., mineral oil and isopropyl myristate) as the receiver vehicle made diffusion experiments difficult to perform and/or assay. For these media, the cellulose release data was not used and D_d was estimated from viscosity data, which gives values that are sufficiently accurate for use here. The basis of this method is the Stokes-Einstein equation (18), given as

$$D = \frac{k_BT}{6\pi\eta r}$$

where k_B is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the liquid medium (donor), and r is the effect radius of the diffusing drug molecule. For

the same drug and temperature, the ratio of the diffusion coefficients in two solvents is given by

$$\frac{D_2}{D_1} = \frac{\eta_1}{\eta_2}.$$

Thus, knowing the diffusion coefficient in one medium D_1, it is possible to estimate the diffusion coefficient in another medium D_2 from the inverse ratio of the viscosities of the two media. In this study, the ratio of viscosities was obtained using a falling ball viscometer method (19) according to

$$\frac{\eta_1}{\eta_2} = \frac{(\rho_B - \rho_1)t_1}{(\rho_B - \rho_2)t_2}$$

where ρ_B is the density of the falling ball, ρ_1 and ρ_2 are the densities of the two solvents, and t_1 and t_2 are the times required for the ball to fall a given distance L through each solvent after achieving its terminal velocity.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

All chemicals were analytical grade or higher in quality. Alpha-tocopherol and alpha-tocopherol acetate were purchased from Sigma Chemical, Co. (St. Louis, MO). Isopropyl myristate NF, light mineral oil, and phosphate-buffered saline ultrapure were from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ). HPLC grade acetonitrile and water were from EM Science (Gibbstown, NJ). Ethanol USP was from AAPER Alcohol and Chemical Company (Shelbyville, KY). Klucel® was from Hercules Inc. (Wilmington, DE).

TOPICAL FORMULATIONS

Three different solutions and two gel formulations, all containing 5% alpha-tocopherol acetate, were used in this study. The solvents of the solutions were ethanol USP, isopropyl myristate, and light mineral oil. The gels consisted of 1% and 3% Klucel®, respectively. Gel formulations were prepared by dispersing Klucel® powder in ethanol USP and mixing by means of a magnetic stirrer to prepare Klucel® gel. Mixing continued until all particles were thoroughly wet. Alpha-tocopherol acetate was weighed accurately and dissolved separately in 95% ethanol USP. Then the alpha-tocopherol acetate solution was introduced dropwise to the Klucel® gel and continuously stirred for four hours at room temperature by means of a magnetic stirrer so that the proper concentration of the gel was achieved.

HPLC ASSAY

Alpha-tocopherol and alpha-tocopherol acetate concentrations were determined by HPLC with a UV-detector according to the method described by Rangarajan and Zatz (20). The chromatographic apparatus consisted of a Hitachi L-7250 programmable auto-

sampler, a Hitachi L-7400 UV detector, and a Hitachi L-7100 pump. The chromatography was performed on a reverse-phase C_{18} (μBondapak™, 3.9 × 300 mm, Milford, MA). The detection wavelength was 285 nm. The mobile phase consisted of acetonitrile:water (96:4). The isocratic flow rate was changed to 2.0 ml/min to reduce the retention time. (The retention times were 10.3 and 12.4 minutes for alpha-tocopherol and for alpha-tocopherol acetate, respectively, compared to 13 and 16 minutes in the source paper.) For both analytes, the peak areas vs concentration (μg/ml) curves were linear in the range of 10–1000 μg/ml. The injection volume was 10 μl.

DIFFUSION STUDIES USING CELLULOSE MEMBRANES

In vitro diffusion studies were carried out using a modified Franz diffusion cell apparatus (Crown Glass Company, Somerville, NJ) with a diameter of 15 mm and a diffusional area of 1.76 cm^2. A Spectra/Por®7 regenerated cellulose membrane (Spectrum, Laguna Hills, CA) was inserted between the donor and the receiving compartment and secured in place by means of a pinch clamp. The membrane had a thickness of 60–65 μm and a molecular weight cutoff point of 1,000. According to the manufacturer's direction, the cellulose membrane was rinsed with distilled water in order to remove traces of the preservative sodium azide before use. The receiving compartment (volume 13.1 ml) was filled with degassed ethanol USP and it was maintained at 37°C by means of a water bath circulator and a jacket surrounding the cell, resulting in a membrane surface temperature of 32°C (18). The receiving medium was continuously stirred by a Teflon™-coated magnetic stirrer, to avoid diffusion layer effects. A 1.00-ml sample of each ATA formulation was accurately measured and placed in the donor compartment and sealed with aluminum foil and parafilm. Aliquots of 0.5 ml were withdrawn from the receiving compartment at 15, 30, and 45 minutes and 1, 2, 3, and 4 hours using a microsyringe, and replaced immediately with an equal volume of degassed ethanol USP. All samples were transferred to 1.5-ml vials and diluted with degassed ethanol USP up to 1.0 ml before analysis by HPLC. The experiment was carried out in triplicate for each formulation. This method was applied to the ethanol solution, 1% Klucel® gel, and 3% Klucel® gel. Where appropriate, the cumulative amount released of ATA vs time data was analyzed and equation 5 was used to calculate the diffusion coefficients of ATA in the formulation. In cases where the slope of the M-vs-t plots was nearly constant, the diffusion coefficient of ATA in the formulation was not calculated exactly, but lower limits for the value were calculated instead using equation 6 with $t = 4$ hours.

RELATIVE VISCOSITY ESTIMATES

The viscosity of ethanol USP, isopropyl myristate, and light mineral oil solvents was determined at 32°C by using the technique of falling ball viscometer. For each solvent measured, 105 ml was poured into a 100-ml graduated cylinder, which was then kept in a water bath for at least 30 minutes before starting the experiment, to ensure that the solvent temperature was 32°C. The plastic sphere ($\rho = 1.0038$ g/cm^3) was carefully placed into the cylinder and allowed to fall through the liquid without touching the wall. The time of descent between the 100-ml and 10-ml marks was timed by means of a stopwatch. The experiments were repeated ten times for each solvent, and the average values were used for further calculation.

IN VITRO CADAVER SKIN PERMEABILITY STUDIES

The same apparatus and experimental procedure described in the previous section were used to perform cadaver skin permeability studies. The sampling times were 2, 4, 6, 8, 12, 24, 30, 36, and 48 hours. The experiment was carried out in triplicate for each formulation. Human cadaver skin from the back of Caucasian subjects was kindly donated by Novartis (Somerville, NJ). Cadaver skin was prepared by hydrating it in an isotonic phosphate-buffered solution for one hour at room temperature before it was placed between the donor and receiving compartments. The integrity of the skin was checked by visual inspection of the cumulative amount-vs-time plots. Experiments in which the amount of permeated ATA suddenly reached a plateau or jumped by large amounts were disregarded and repeated.

STATISTICAL DATA ANALYSIS

Permeability calculations were done by nonlinear regression, using the Solver function in Microsoft Excel™. Other statistical analyses were performed with the SPSS 10.1 for Windows 2000 version using one-way analysis of variance (ANOVA). Analyses were performed primarily to determine whether there were any significant differences in alpha-tocopherol acetate release among the different concentrations of Klucel® in the formulations and between each solution formulation.

RESULTS

DIFFUSION COEFFICIENTS OF ATA IN THE DONOR MEDIA

The release profiles of ATA from the ethanol solution, 1% Klucel® gel, and 3% Klucel® gel through regenerated cellulose membrane were analyzed using equations 5 and 6. The diffusion coefficients of ATA in the formulation were 2.5×10^{-2} cm^2/h for the ethanol solution, and greater than 1×10^{-2} cm^2/h for the 1% and 3% Klucel® gels, respectively. The ratios of solvent viscosity were used to calculate diffusion coefficients of ATA for the isopropyl myristate solution and the light mineral oil solution. The ethanol/isopropyl myristate viscosity ratio was 0.91 and the ethanol/light mineral oil viscosity ratio was 0.70, and the estimated diffusion coefficients of ATA in isopropyl myristate and light mineral oil were 2.3×10^{-2} cm^2/h and 1.8×10^{-2} cm^2/h, respectively.

CADAVER SKIN PERMEABILITY

The permeability coefficient of ATA though human cadaver skin was calculated using equation 1, which applies when the concentration profile in the membrane develops relatively slowly and diffusion through the membrane is the rate-controlling step. Figure 1 shows the experimental data for the cumulative amount released as a function of time for the five formulations, and the model fits the data. The calculated permeabilities through human cadaver skin membrane were $1.0 \times 10^{-4} \pm 1.5 \times 10^{-5}$ cm/h for the ethanol solution, $1.1 \times 10^{-2} \pm 1.6 \times 10^{-3}$ cm/h for the isopropyl myristate solution, $1.4 \times 10^{-4} \pm 3.8 \times 10^{-6}$ cm/h for the light mineral oil solution, $2.1 \times 10^{-4} \pm 2.6 \times 10^{-5}$ cm/h for 1% Klucel® gel, and $4.7 \times 10^{-4} \pm 4.6 \times 10^{-5}$ cm/h for 3% Klucel® gel.

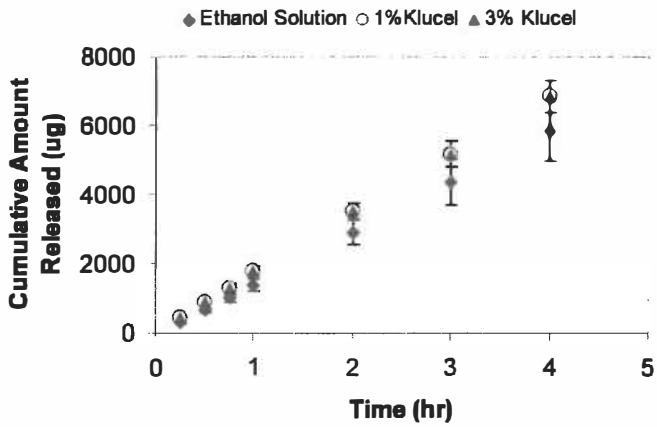

Figure 1. Average (n = 3) cumulative amount of ATA ± standard deviation found in the receiver compartment of modified Franz cells for the regenerated cellulose membrane experiments.

ANOVA performed on the cumulative ATA amount found in the receiver at each time point showed that the isopropyl myristate formulation produced a significantly larger permeation of ATA through the skin than the other formulations ($p > 0.001$). There was no difference in the cumulative amount detected among the other formulations. Therefore, it can be concluded that 1% and 3% Klucel® or ethanol solution and light mineral oil solution without Klucel® did not make any difference in the permeation of ATA through the skin. Small amounts of AT were detected only in some studies and did not increase with time, suggesting that the AT detected was not a metabolite of ATA but may be attributed to the AT already present in the human cadaver skin.

DISCUSSION

In this study, the permeation through human cadaver skin of ATA was determined using a new mathematical approach. The method used a modified Franz cell apparatus and 95% degassed ethanol in the receiver compartment. Previous ATA permeability studies (14) used Dulbecco's modified phosphate-buffered saline to maintain skin viability and 3% bovine serum albumin to improve ATA solubility, as reported in the literature (19). However, the study reported that the ATA collected in the receiving medium of the Franz cell was always negligible (below detection limits). Another study (21) reported the use of phosphate buffer and Tween 80 as a receiving medium, and again it failed to show ATA in the receiving compartment. The goal of the present study was to find an experimental setup that allowed a fast and relatively accurate method to evaluate the permeability properties of different formulations of ATA across the skin.

The mathematical method used (16) requires sink conditions to work. Therefore, the solubility of ATA in the receiver media is a key part of the experiment, even if it compromises the viability of the skin. Solubilization of ATA in Dulbecco's buffer with an increasing percent of ethanol provided unsatisfactory results. Finally, it was decided to use 95% ethanol because ATA is freely soluble in this media. However, the use of a lipophilic receptor fluid has the potentiality to extract lipids from the skin barrier and

to artificially increase skin permeability (22). Because this artifact would be common to all the formulations tested in this experiment, it would not affect the relative results.

Previous studies showed that ATA is slowly metabolized in the viable part of the skin (20). Therefore, if some metabolic capabilities were left in the skin, AT would be detected in the receiver container. For this reason, the samples collected from the receiver container were analyzed with an assay able to quantify both ATA and AT. However, AT was detected only in a few studies and it didn't increase with time. It is then possible that the AT detected was not a metabolite of ATA but came from AT content already existent in the human cadaver skin.

ATA was detected in the receiver medium in a time-dependent way, and cumulative-amount perfused curves could be built (Figure 2). The mathematic model proposed by Bellantone *et al.* (16) was used in this study because the donor compartment is unstirred. A popular method to estimate skin permeability *in vitro* is the lag-time method (23), which requires that the drug be held at constant concentration in the donor compartment. However, that method requires a constant concentration at the donor–membrane interface. Because in our experiment the donor is unstirred and possibly substantial depletion of drug occurs due to drugs that cross the skin, the concentration in the donor is not constant and the lag-time analysis does not apply. Figure 3 shows the model fits obtained from the lag-time method (gray line) and equation 1 (black line) for one set of experimental data. It can be seen that equation 1 has a more natural and accurate fit than the lag-time model. In addition, it has been pointed out (16) that the method gives accurate values for the permeability, using experiments that are usually considerably shorter (by half or one-third) than those required by lag time or other steady-state approximation methods.

The mathematical model used in this paper to estimate permeability through human cadaver skin (membrane is the rate-limiting step) requires a previous estimate of the diffusion coefficient in the donor compartment D_d. For the ethanol solution and the two gel formulations, D_d was estimated by permeability studies through a regenerated cellulose membrane. This method was considered appropriate because the similarity between the medium of the donor compartment and the receiver compartment would minimize backflow of fluid to the donor, which could significantly decrease the net release of the drugs into the receiver. A value for D_d was obtained for the ethanol donor, and lower limits for D_d were obtained for the alcoholic gels. The same approach was attempted for isopropyl myristate and light mineral oil by filling the receiver compartment with isopropyl myristate and light mineral oil, respectively. However, the experiment was technically difficult because isopropyl myristate and light mineral oil, used as receiving medium, could not be injected directly into the HPLC. By UV spectrophotometer analysis, the concentration of ATA in the receiver was always below the limit of detection. Hence, the diffusion coefficients of ATA from the isopropyl myristate and light mineral oil solutions were estimated by measurement of the viscosity of the solvents and by calculations.

The results of these studies show that, under the experimental conditions used, the formulation of ATA in isopropyl myristate has a permeability though human cadaver skin that is two orders of magnitude larger than those of the other formulations. A possible explanation for this finding is that isopropyl myristate has a blend of polar and non-polar properties, which probably mimic to some extent the complex lipid/polar

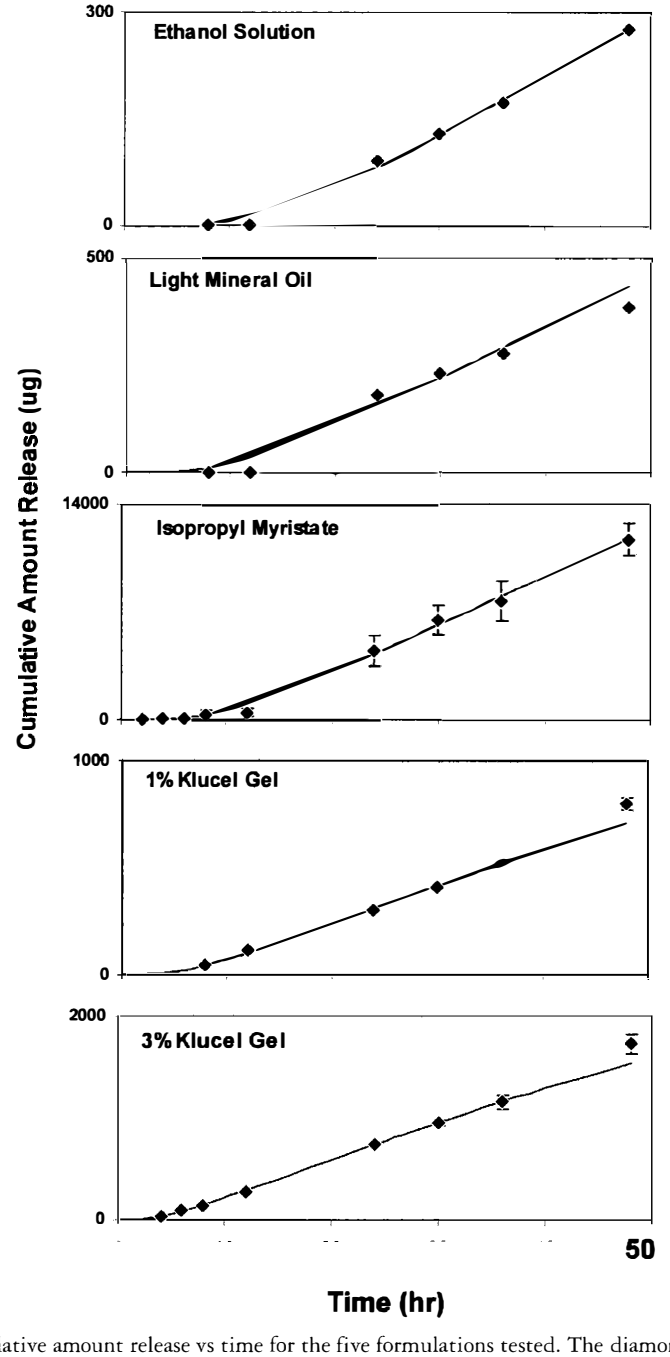

Figure 2. Cumulative amount release vs time for the five formulations tested. The diamonds (♦) represent the experimental data and the solid line the fit using equation 1. The error bars represent ± the standard deviation (n = 3). For the ethanol and mineral oil solutions the error bars are too small to be seen.

nature of the stratum corneum (11). This property would make isopropyl myristate thermodynamically similar to the stratum corneum and would facilitate the permeation of ATA through the skin. It is also known that water, which is present at the surface of

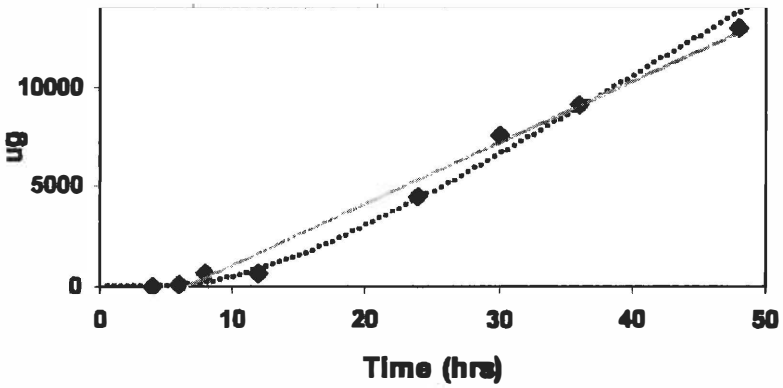

Figure 3. Cumulative amount released vs time for the diffusion of ATA from isopropyl myristate through human cadaver skin. Diamonds (◆): experimental data. Dashed black line: fit by model case 1. Solid gray line: fit by the lag-time model.

the skin and in the skin itself, can create a resistant boundary at the donor–skin interface and may prolong or delay the permeation of poorly water-soluble molecules. ATA has to dissolve in this water layer before reaching the skin, and the presence and thickness of this layer would affect the permeability. For the alcoholic gels, it is possible that the gelling agent absorbs the water present on the skin membrane and therefore reduces the time to permeate into the skin, as can be seen from the shorter lag time as the percent of the Klucel® agent increases (12, 8, and 2 hours for the ethanol solution and the 1% and 3% Klucel® formulations, respectively). However, reduction of the water layer is not sufficient to promote substantial penetration into the skin. ATA released from the ethanol solution and 1% and 3% Klucel® didn't differ significantly, as shown in Figure 2 or by the values of the permeability coefficients. The light mineral oil solution has a permeability similar to that of the ethanol formulations. It is possible that the layer of water at the interface between the formulation and the skin would be thicker because of the incapability of mineral oil to absorb it, and it would contrast the facilitating effect due to the similarity between the lipid bilayer of the stratum corneum and the light mineral oil.

CONCLUSION

Permeability studies performed on five different formulations of ATA show that isopropyl myristate favors the highest permeability through human cadaver skin in the experimental conditions tested in this study. There was not a statistically significant difference between the permeabilities of the other formulations, suggesting that the formulation had relatively minor effects on the permeation of ATA. Further studies are necessary to confirm these findings *in vivo.*

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