J. Cosmet. Sci., 54, 161–174 (March/April 2003)

Effect of formulation on the topical delivery of α-tocopherol

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Accepted for publication August 19, 2002.

Synopsis

The objective of this research was to investigate the effect of concentration and delivery system on skin permeation of α-tocopherol (α-T). Also, the addition of sunscreens and oleic acid on α-T permeation was studied using an *in vitro* micro-Yucatan pig skin model. Various delivery systems of α-T (1%) were formulated, which included simple solution, gels, emulsions, and microemulsions. The experimental design chosen for this study was a statistical randomized complete block design. α-T delivery was proportional to its concentration. The hydroalcoholic gel delivered significantly higher amounts of α-T into the receptor than the other gels used. A microemulsion containing isopropyl myristate emerged as the best delivery system for α-T among all the systems studied. Pig skin is a suitable *in vitro* model for studying the permeation of α-T and possibly other antioxidants, though *in vivo* experiments in humans are required to further corroborate the data.

INTRODUCTION

Research has shown that UV radiation damages DNA and genetic material, oxidizes lipids and produces harmful free radicals, causes inflammation that also produces free radicals, disrupts cell communication, causes expression of stress response genes, and weakens the immune response of the skin. α-Tocopherol (α-T) is the major lipophilic antioxidant in many biological systems (1). Topical application of α-T has been shown to protect against UV-induced cutaneous damage, carcinogenic and mutagenic activity of ionizing radiation, and chemical agents (2). α-T has been found to reduce tumor incidence in mice (3) and decrease fine lines, wrinkles, and sagging induced by photoaging (4,5). α-T is now considered essential for the stabilization of biological membranes, particularly those containing large amounts of polyunsaturated fatty acids. The oxidation of unsaturated fats produces lipid peroxides, which interfere with the structure and function of biological membranes. When *dl*-α-tocopherol was added to commercial

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creams in concentrations of 0.1% or 1%, no marked increase in lipid peroxides was observed during a 96-hour exposure to sunlight (6). The antioxidant activity of α-tocopherol has been summarized elsewhere (7).

Varying concentrations of α-T have been used in different studies. Beijersbergen van Henegouwen *et al.* (8) have used 0.25% of α-TAc to study its UV protecting activity *in vivo* in rat. α-T in low concentrations of 0.05–2% was used as an antioxidant in topical preparations. In higher concentrations (2–10%) α-T seemed to have benefit in stratum corneum hydration (9,10). Pure α-T and concentrated α-T preparations (10–20%) could irritate human skin, and cases of allergic contact dermatitis and other types of immunologic hypersensitivity reactions are reported (11). However, there is no systematic study in the literature on the effect of concentration of α-T on skin permeation.

Sunscreens are the second type of photoprotectants that are commonly used along with antioxidants. Very little information, however, is available on the augmentation of the activity of either the sunscreens in the presence of antioxidants or vice versa. Darr *et al.* (12) studied the combination of the two antioxidant vitamins, C and E, with commercial UVA sunscreen (oxybenzone) and found greater than additive protection against phototoxic damage. These results emphasize the importance of combining antioxidants with common sunscreens to maximize photoprotection. Alberts *et al.* (13) reported that in a survey about 62% of sunscreen formulations contained some form of α-T. They caution, however, that further research has to be performed to study the potential harmful effects of "other ingredients" (like α-T) that can be added to commercial sunscreens. Alteration of the skin transport of chemicals can be achieved by means of permeation-enhancing agents such as oleic acid and dodecylazacycloheptan-2-one (laurocapram, azone) (14).

Bhatia *et al.* (15) used a combination of ethanol and 10% oleic acid pretreatment to significantly increase the permeability coefficient of cholecystokinin-8. Kitagawa and Li (16) found that 1% 1-menthol plus 15% ethanol increased the permeability of benzoic acid but decreased that of its higher alkyl substituents. Morgan *et al.* (17,18) studied a new class of penetration enhancers, octyl salicylate (OSal) and padimate O (Pad O), which have been used as effective sunscreens, along with oleic acid, to enhance the permeability of the sex hormones testosterone, estradiol, and progesterone. They found OSal to give the highest enhancement in permeability. The effects of padimate O and oleic acid were comparable to each other, albeit better than the control.

Previous work done by the authors using the prodrug ester of α-T, α-tocopheryl acetate (α-TAc), demonstrated the metabolism of α-TAc to α-T in pig skin (19). Permeation of α-TAc and its metabolism were found to be a function of the delivery system (20). An emulsion system containing isopropyl myristate (IPM) emerged as the most desirable formulation in terms of skin delivery of α-TAc.

The objective of this work was to (i) formulate different delivery systems for α-T, (ii) delineate the effect of both biphasic and uniphasic delivery systems on the permeation of α-T, (iii) study the effect of concentration on α-T permeation, (iv) characterize the effect of sunscreens on α-T permeation, and (v) formulate and study α-T permeation with oleic acid, which is believed to function as a penetration enhancer.

MATERIALS AND METHODS

CHEMICALS AND INSTRUMENTS

D-α-Tocopherol was obtained as a gift from Archer Daniels Midland Company (IL).

The following chemicals were obtained directly from the manufacturer and used without purification: SD alcohol, Eastman (TN); isopropyl myristate (IPM) and mineral oil, Sigma Chemical Company (NJ); diisopropyl adipate (DIA), Ceraphyl® 230, isocetyl alcohol, and Ceraphyl®, ISP Vandyk (NJ); carbomer, Carbopol®, B F Goodrich (OH); DEA-cetyl phosphate, Roche Vitamins and Fine Chemicals (NJ); diazazodinyl urea and germall, Sutton Laboratories (NJ); ethomeen C/25, Akzonobel (IL); hydroxypropyl cellulose, Klucel®, Hercules Co., Germany; Tween®20, polyoxyethylene (20) sorbitan monolaurate, and Tween®80, polyoxyethylene (20) sorbitan monooleate, ICI Surfactants (DE); and Transcutol, diethylene glycol monoethyl ether, Gattefosse (France). Scintillation fluid Scintiverse, reagent alcohol, and glacial acetic acid were of HPLC grade and were obtained from Fisher Scientific (Springfield, NJ). Water refers to freshly deionized water. Tissue solubilizing fluid, Solvable™, was purchased from Packard Instrument Co. (Meriden, CT). Transparent tape # 800 was purchased from 3M Packaging Systems Division (St. Paul, MN). All other materials were obtained from standard sources.

TOPICAL FORMULATIONS

Formulations for α-T permeation study. All formulations were prepared on a weight/weight basis. The compositions of the formulations used in this study are shown in Tables I and II. Gels 1, 2, and 3 differed in their alcohol content. Gels 1 and 2 were purely alcoholic, with gel 2 containing a surfactant. Gel 3 was a hydroalcoholic gel. The oils in the case of the macroemulsion formulation (emulsion 1) were kept at a constant ratio of diisopropyl adipate:mineral oil, 1:1, throughout the experiment. Emulsions were made by heating the oily and aqueous phases separately (55°C) and shear mixing the two phases (aqueous added to oily) to yield creamy emulsions. Simple vortexing was sufficient to give clear microemulsions.

Formulations for concentration study. The concentrations chosen for the concentration dependency study were 0.25%, 1%, and 4% α-T. Prototype formulations including the IPM solution, gel 3, and emulsion 1 were made at these three concentrations of α-T. Higher or lower amounts of α-T were compensated for by decreasing or increasing the solvent for α-T used in the corresponding 1% formulations (IPM for solutions, SD alcohol for gels, and the oils for emulsions).

Formulations with sunscreens. Sunscreen formulations containing α-T included gel 3 and emulsions 1 and 3. Two sunscreens were studied, octyl methoxy cinnamate (OMC) and octyl salicylate (OSal), at concentrations of 7% and 5%, respectively. The formulations

Table I
Topical Gel Formulations Used in the Study: α-Tocopherol Gels

Ingredient	Gel 1 (% w/w)	Gel 2	Gel 3
α-T	1	1	1
SD alcohol	96	91	69
Isocetyl alcohol	—	—	14
PEG-15 cocamine	—	5	5
Hydroxy propyl cellulose	3	3	3
Water	—	—	8

Table II
Topical Emulsion Formulations Used in This Study

Ingredient	α-T (% w/w)
Emulsion 1[a]	
α-T	1.00
Diisopropyl adipate	5.38
Mineral oil	5.38
DEA-cetyl phosphate	2.00
Diazazodinyl urea	0.30
Carbomer	0.30
Water	85.64
Emulsion 2[b]	
α-T	1
Isopropyl myristate	10
Polysorbate 80	16
Sorbitol	30
Water	43
Emulsion 3[c]	
α-T	1
Benzyl alcohol	21.3
Diethylene glycol monoethyl ether	16.9
Tween 20	18.1
Water	42.7

[a] o/w macroemulsion.
[b,c] Microemulsion containing IPM or benzyl alcohol as oily phases.

remained similar to gel 3 (Table I) and emulsions 1 and 3, given in Table II, with the difference of the added sunscreen agents. The added ingredients were compensated for by a decrease in the concentration of SD alcohol in the gels and the oily phases in the microemulsions. The ratio of the two oils for emulsion 1 was still maintained the same.

Oleic acid study. For the penetration enhancer study, the IPM solution, emulsion 1, and gel 3 were studied, containing in addition to the ingredients listed in Table I and II, 1% oleic acid. A gel 3 formulation containing 5% oleic acid was also included for study purposes. A control experiment with α-T was run simultaneously with all three formulations without the added oleic acid.

All α-T formulations were observed for stability for a period of two weeks. Also, to ensure that α-T was not oxidized during manufacture itself, cold formulations immediately upon manufacture and two weeks later were assayed for drug content by HPLC. Details of the HPLC procedure are given elsewhere (19).

RECEPTOR FLUID

The receptor fluid was an aqueous solution of 0.1% polyoxyethylene oleyl ether (PEG-20 oleyl ether), a nonionic surfactant with an HLB of 16. This receptor fluid, though not physiologic, maintains adequate solubility of α-T without affecting skin barrier function (21). The receptor was pumped at a rate of 1.5 ml/h. The apparent solubility of α-T in 0.1% PEG-20 oleyl ether at 37°C was measured to be 1.08 mg/ml, substantially in excess of the maximum concentration in the receptor solution, thereby assuring maintenance of the sink condition at all times.

TISSUE AND PREPARATION

Micro-Yucatan pig skin obtained from Charles River Laboratories (Wilmington, MA) was used as the biological membrane to study *in vitro* percutaneous absorption. Upon receipt the freshly excised skin was washed gently with 1% (w/w) aqueous detergent, rinsed with deionized water, and patted dry with a paper towel. A 250–300-μm-thick layer of the skin was cut from the surface with a Padgett Electrodermatome™ instrument (Padgett Instrument, Kansas City, MO). The skin pieces were then rinsed and dried with paper towels before storage in plastic bags at 4°C. Skin was removed from the refrigerator and kept in isotonic solution to hydrate at room temperature one hour before starting the experiment. The dermatomed skin was cut into 10-mm circular pieces with a brass punch and placed epidermis-side-up in Bronaugh diffusion cells. The skin treated in this fashion from the stage of receipt until use retained its original permeability characteristics for four weeks after dermatoming (22).

RADIOLABELING OF α-T

D-alpha-[^{3}H]tocopherol was custom synthesized by Amersham Pharmacia Biotech, England. This was received as a toluene:ethanol (9:1) solution with a specific activity of 19 Ci/mmol (molecular weight 432, at this specific activity). α-T formulations were spiked with the radiolabeled α-T such that each finite dose of 5-μl formulation applied on the skin contained approximately 300,000 dpm (disintegrations per minute). Gels were spiked before adding the gelling agent. Biphasic o/w formulations were spiked before addition of the aqueous phase. Analyses for the radiolabeled α-T throughout this experiment were carried out with a liquid scintillation counter (LSC, Beckman Instruments).

STATISTICAL DESIGN OF THE EXPERIMENT

The application of formulations on the pig skin was carried out using a statistically approved model. A randomized complete block design was chosen as the design for the experiment. The statistical model appeared as shown in Table III. For the other studies, the application of each formulation was completely randomized over the number of cells and days of the study.

DOSING

Finite dosing was used to simulate the actual use conditions in all the *in vitro* permeation and metabolism experiments. The smallest volume of the formulation required, to obtain complete and uniform coverage of the diffusion cell surface area (0.636 cm^2), was determined to be 5 μl, corresponding to a weight of about 4 mg for each formulation. After application, the preparation was uniformly spread on the stratum corneum (SC) side of the skin with the help of a glass rod, and the tip of the rod was washed into a vial containing 1.5 ml of ethanol in order to account for the material lost on spreading. With this technique, the exact amount of material applied to the skin surface was determined. To estimate the amount of α-T in the original formulation, 5 μl of the

Table III
Statistical Randomized Complete Block Design for the Application of Formulations

Formulation	Day[a] 1	Day 2
IPM solution	W1[b]	Y1[d]
	X1[c]	Z1[e]
Gel 1	W2	Y2
	X2	Z2
Gel 2	W3	Y3
	X3	Z3
Gel 3	W4	Y4
	X4	Z4
Emulsion 1	W5	Y5
	X5	Z5
Emulsion 2	W6	Y6
	X6	Z6
Emulsion 3	W7	Y7
	X7	Z7

[a] Two consecutive days, 1 and 2.
[b,c] Replicates on day 1 for IPM solution.
[d,e] Replicates on day 2 for IPM solution.

formulation was counted for its radioactive counts after equilibration of each formulation for a period of 24 hours.

IN VITRO SKIN PERMEATION/METABOLISM METHODOLOGY

A flow-through system was used for conducting *in vitro* permeation experiments. The total system consisted of a receptor fluid reservoir; a variable flow rate peristaltic pump, Cassette® (Manostat, New York); a circulating water bath, Lauda® (Brickman Instrument, Westbury, NY); and a two-cell-holding heating block, 14 Teflon® flow-through diffusion cells, and a Retriever IV fraction collector (ISCO Inc., Lincoln, NE) to collect effluent fractions over the adjusted time period. Each diffusion cell had an inner diameter of 9 mm and a surface area of 0.636 cm^2 exposed to the receptor fluid. The receptor fluid was pumped at a flow rate of 1.5 ml/h from the reservoir to the diffusion cells placed in the holding blocks. The skin surface temperature was maintained at 32°C by adjusting the circulating water bath temperature to 39°C (23). The effluent from the diffusion cells was collected directly into glass scintillation vials every four hours for a period of 24 hours.

SKIN TREATMENT

The liquid scintillation counting technique was used to analyze all the *in vitro* permeation samples. In each experiment a minimum of four replicates were used. At the conclusion of the experiment, scintillation fluid was added to the effluent samples collected directly into the vials and the amount of α-T penetrated was estimated from the counts of radioactivity present in the samples. Counts were obtained as dpm, which were then converted into micrograms of active by taking into account the spiking ratio for each formulation and specific activity of the active.

After 24 hours the donor compartment was washed three times with 1 ml of acetonitrile. Washes were collected and analyzed for the amount of α-T remaining on the skin surface. Washed skin samples were removed from the cells. The tape-stripping technique was used to separate the SC from the rest of the epidermis to get an estimate of material remaining in the barrier layer of the skin. In this technique, seventeen strips of the active-treated side of the skin, using a 3M Scotch™ tape, were taken as two + fifteen strips. The first two strips represented the active superficially adhering to the surface (and so were included in the wash) and the next 15 strips represented the active recovered from the SC. Scintillation fluid was added to the vials containing the tape strips and allowed to stand at room temperature for at least 24 hours to enable extraction of α-T in order to perform scintillation counting on the samples. The remainder of the skin was digested in 3 ml of tissue solubilizer at 50°C for 24 hours in an oven. This was done to get an estimate of material in the viable tissues of the skin. After skin digestion, the samples were neutralized with glacial acetic acid, and scintillation fluid was added for counting.

Receptor solution was collected in glass scintillation vials every four hours. Scintillation fluid was added to the vials and they were counted 24 hours later. Thus, the amount of α-T was estimated in the following four locations in the *in vitro* permeation experiment: (a) receptor fluid, (b) washes, (c) stratum corneum, and (d) viable tissues of the skin.

STATISTICAL ANALYSIS

Statistical differences between the formulations and all the other formulations with the simple solution were estimated using a Student's *t*-test. In the oleic acid study, the results with the oleic acid were compared to the control formulations, which did not contain any oleic acid. The design of these studies enabled us to compare α-T permeation with α-tocopheryl acetate (α-TAc) permeation in a study that was published earlier (19).

RESULTS

COMPARISON OF VEHICLES

Figure 1 shows the permeation of α-T in terms of the amount of active in the stratum corneum, the viable skin, and the total amount permeated, which is inclusive of the amount of α-T in the SC, viable skin, and receptor. IPM solution yielded significantly higher amounts of α-T ($\alpha = 0.05$) in the SC than gels 1 and 2 and emulsion 1. The other formulations did not differ from each other in terms of this parameter. In the viable skin, emulsion 2 had higher permeation, which differed significantly from that of emulsion 3 ($\alpha = 0.05$). IPM solution had a higher amount of total α-T permeation than emulsion 3 and gel 1. However, this amount was significantly lower than the amount permeated with emulsion 2. Emulsion 2 had the highest permeation compared to all the other formulations. It was significantly higher than for the other emulsion formulations, IPM solution, and gel 1. Gel 3 also had significantly higher total permeation compared to gel 1 in this study. In terms of the amount of α-T in the receptor at 24 hours, gel 3 fared the best, having significantly higher permeation compared to the other gels.

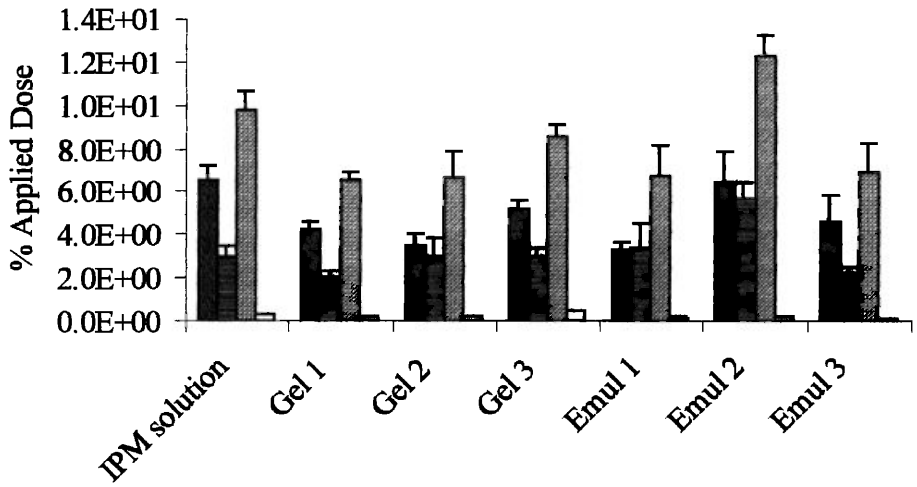

Figure 1. Effect of formulations on the skin delivery of α-T. Values are percent applied dose ± SEM (n = 4). ■, Stratum corneum. ▤, Viable skin. ▨, Total amount permeated. □, Amount in receptor.

Comparison of α-T and α-TAc permeation. Results of α-T permeation study were compared to α-TAc permeation studies performed earlier (19). α-TAc is a commonly used prodrug precursor of α-T and it has to undergo metabolism by skin enzymes to release the active antioxidant, α-T. Table IV shows the comparative values for the permeation in viable skin and receptor and total skin and receptor for α-T and α-TAc, as a function of the delivery system. α-T permeation studies showed a lower permeation of active than the α-TAc studies through all the parameters measured. To find the extent of this decrease, a ratio was taken of the average amount of α-TAc permeated to the α-T permeated. The amount of active permeated in viable skin and receptor in the α-T permeation study was lower by about 2.22 times than the active that permeated from the α-TAc permeation study. The α-TAc permeation study had values about 2.98 times higher than the α-T study when measured as total skin plus receptor, which is inclusive of the stratum corneum. Although the values obtained in studies using either α-TAc or α-T were numerically different, certain trends observed in both the studies remained the same. For

Table IV
Comparison of α-T and α-TAc Permeation at 24 Hours

	Viable skin + receptor (% applied dose)			Total skin + receptor (% applied dose)		
Formulation	α-T	α-TAc	α-TAc/α-T	α-T	α-TAc	α-TAc/α-T
IPM solution	9.74 ± 0.89	15.93 ± 2.18	1.64	3.17 ± 0.49	6.66 ± 1.27	2.10
Gel 1	6.47 ± 0.54	17.54 ± 4.79	2.71	2.24 ± 0.24	8.73 ± 3.19	3.89
Gel 2	6.62 ± 1.26	9.99 ± 0.95	1.51	3.16 ± 0.88	6.42 ± 0.26	2.03
Gel 3	8.55 ± 0.55	19.69 ± 2.28	2.30	3.42 ± 0.37	9.65 ± 1.10	2.82
Emulsion 1	6.72 ± 1.4	16.02 ± 4.27	2.38	3.46 ± 1.22	9.85 ± 1.98	2.85
Emulsion 2	12.24 ± 0.98	31.67 ± 5.87	2.59	5.83 ± 0.77	15.22 ± 2.27	2.61
Emulsion 3	6.95 ± 1.28	16.82 ± 4.97	2.42	2.30 ± 0.32	10.49 ± 3.10	4.56
Average			2.22			2.98

Values are percent applied dose ± SEM (n = 4).

example, in terms of the total amount of active permeated in both the studies, permeation from emulsion 2 was statistically significantly greater than from emulsions 1 and 3, whereas emulsions 1 and 3 did not differ from each other. In terms of the amount permeated in viable skin and receptor, again emulsion 2 emerged as the best formulation. Permeation of active was significantly higher compared to emulsion 3 in the α-T permeation and compared to the solution in the α-TAc permeation. There were no differences between the other formulations in both the studies. Thus, although the two sets of experiment were conducted at different times and under different experimental conditions, using different analytical techniques (HPLC for the α-TAc assay and LSC for the α-T assay), a similar trend in permeation as a function of formulation was observed. There is very little structural difference between α-TAc and α-T. They have similar lipophilicities and are devoid of functional groups, which can dissociate to give different ionic forms at skin pH. They may be expected to behave similarly with respect to skin penetration (7).

CONCENTRATION STUDY

Representative receptor profiles for the concentration dependency study of IPM solution are shown in Figure 2. The linearity of the plots suggests the achievement of steady state. The slopes of these straight lines, which are the flux values, along with their r^2 values for all three formulations, are shown in Table V. As the concentration increased, there was a proportional increase in flux, showing the existence of dose proportionality for α-T permeation. Interestingly the permeation values for the active in the stratum corneum at different concentrations showed a deviation from linearity, which was unique to the stratum corneum and not observed in the permeation profiles for the rest of the skin tissue. Figure 3 shows the permeation of α-T in viable skin when the amount permeated was plotted as $\mu g/cm^2$.

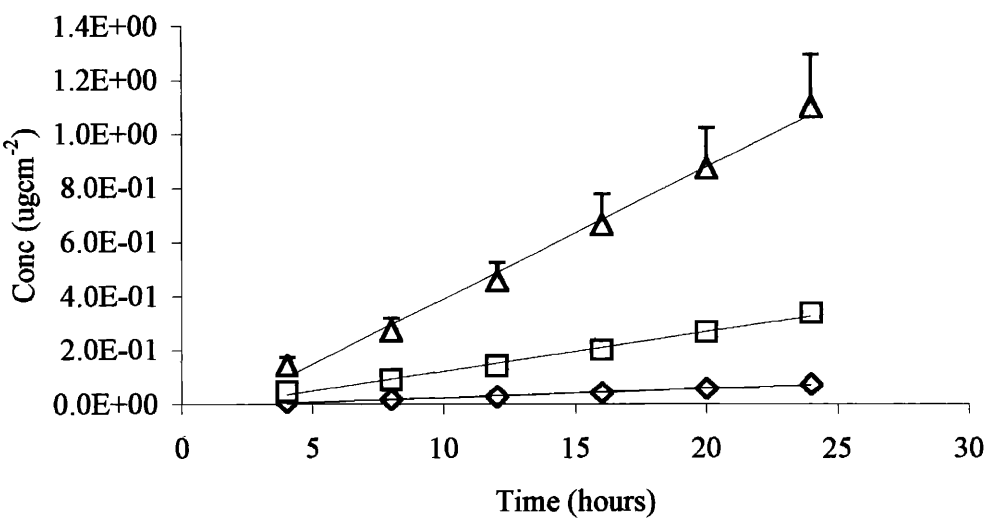

Figure 2. Plot of receptor profiles for IPM solution in the α-T concentration study. Values are concentration ($\mu g/cm^{-2}$) ± SEM (n = 4). Equations of the straight lines are shown. ◇, 0.25% IPM solution. □, 1% IPM solution. △, 4% IPM solution. Regression equations: y = 0.0033x - 0.0084, r^2 = 0.9899; y = 0.0146x - 0.0242, r^2 = 0.9919; and y = 0.0485x - 0.0946, r^2 = 0.993.

Table V
Slopes and r^2 Values for the Receptor in α-T Concentration Study

	IPM solution		Gel		Emulsion	
Concentration	Flux	r^2	Flux	r^2	Flux	r^2
0.25	0.0033	0.990	0.0041	0.999	0.0012	0.994
1	0.014	0.992	0.011	0.984	0.0065	0.993
4	0.0485	0.993	0.0397	0.960	0.0327	0.980

Flux values are in $\mu g/cm^{-2}hr^{-1}$.

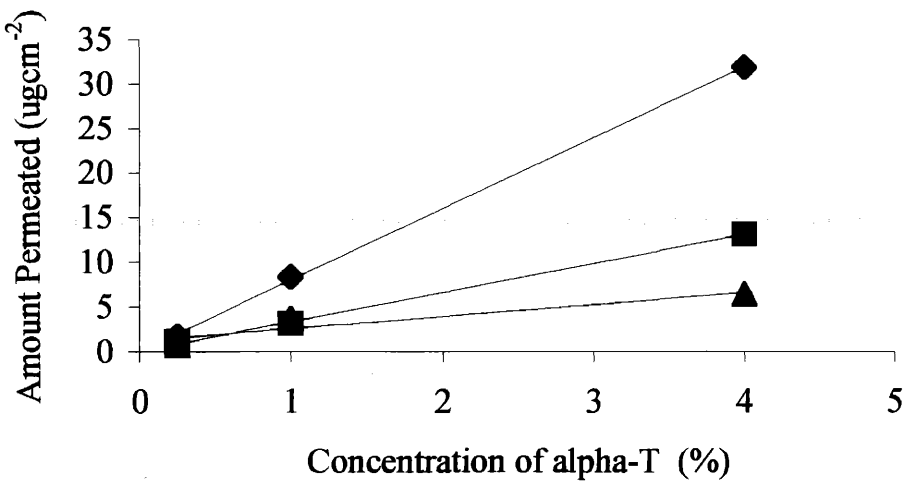

Figure 3. Permeation profile of α-T in viable skin for the concentration study when expressed as $\mu g/cm^2$ ± SEM (n = 4). ◆, IPM solution. ■, Gel 3. ▲, Emulsion 3. Regression equations: y = 7.9944x + 0.0225, r^2 = 0.9997; y = 3.2494x + 0.1042, r^2 = 0.999; and y = 1.3517x + 1.2034, r^2 = 0.8756.

EFFECT OF SUNSCREENS

The formulations included in this study were gel 3, emulsion 1, and emulsion 3 for two different sunscreens, OMC and OSal. Figure 4 shows the profile of sunscreens in viable skin and the total amount permeated, which refers to the amount of active in the stratum corneum, viable skin, and receptor, and the amount permeated into the receptor as % applied dose. Also included in the same graph are the values for the α-T emulsion 1, gel 3, and emulsion 3, which are the control formulations devoid of sunscreens. Emulsion 1 containing sunscreens did not show any significant difference in permeation compared to the control. However, both the gels bearing the sunscreens delivered higher amounts of α-T in the receptor. In general, there was no significant difference between formulations containing sunscreens and control formulations.

EFFECT OF OLEIC ACID (OA)

The formulations used in the study were IPM solution with and without oleic acid, gel 3 with and without oleic acid, and emulsion 1 with and without oleic acid. Also included was a 5% oleic acid gel 3 to study the effect of increasing oleic acid concen-

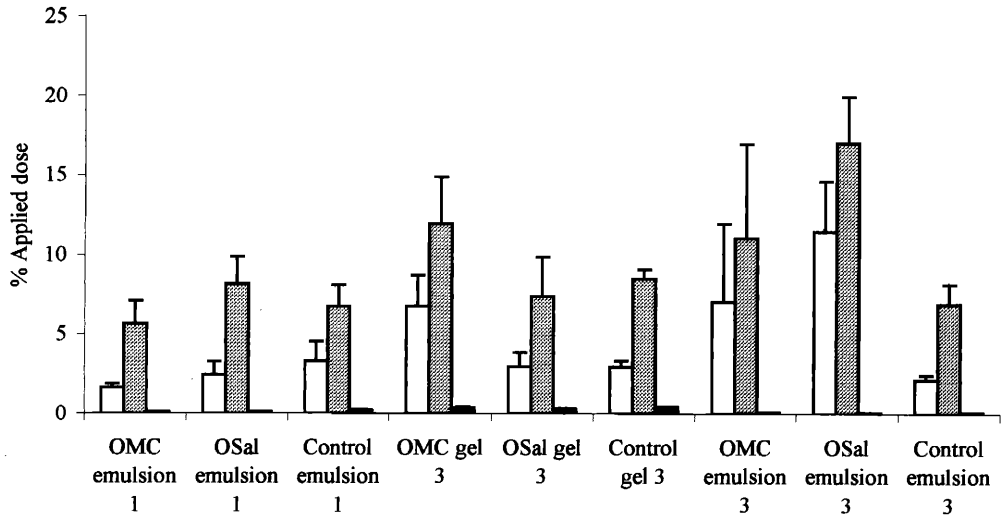

Figure 4. Profiles for sunscreen formulations. Values are percent applied dose ± SEM (n = 4). □, Amount of α-T in viable skin. ▨, Amount of α-T permeated in SC + viable skin + receptor. ■, Amount of α-T permeated in receptor.

trations. Figure 5 shows the profile for α-T permeation in the skin from oleic acid and control formulations. Included are values in the viable skin and receptor and the total amount permeated, which have been described before. Oleic acid formulations were compared to control α-T formulations using a Student's *t*-test (α = 0.05). The 5% oleic acid gel 3 was compared with both the 1% oleic acid gel 3 and with control α-T gel 3. In each case no statistically significant difference was obtained between the test and control formulation at α = 0.05.

DISCUSSION

This study shows that α-T can be delivered into viable skin and receptor fluid from various formulations. Topical α-T treatment results in a significant increase in α-T

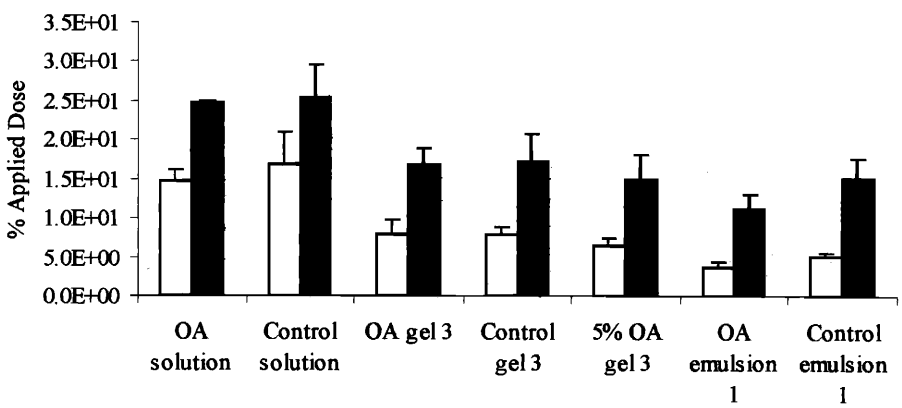

Figure 5. Skin profiles for oleic acid study. Values are percent applied dose ± SEM (n = 4). □, Viable skin + receptor. ■, SC + viable skin + receptor.

concentrations in the viable skin, the stratum corneum, and the receptor fluid. Earlier studies (24) had shown the absence of detectable amounts of baseline α-T in pig skin without any exogenous application, using an HPLC equipped with a UV detector (detection limit 0.25 μg/ml). Traber *et al.* (25) studied the penetration and distribution of α-T applied on mouse skin. They found that the largest fraction of skin α-T following topical application was not found on the surface but in the deeper subcutaneous layers. We also found that topical α-T application markedly increased skin α-T content. Lopez-Torres *et al.* (26) found topical α-T treatment to significantly increase α-T levels in both the epidermis (62-fold) and the dermis (22-fold) 24 h after administration. Two routes of skin absorption of α-T have been suggested: from the stratum corneum into the epidermis and dermis and through the hair follicles by way of the pilosebaceous canal and into the outer root sheaths and eventually into the dermal tissue (27).

This is the first systematic study delineating the effect of formulation factors on the permeation of α-T. All our formulations were studied for stability using a previously established HPLC procedure immediately after formulation and two weeks of storage under ambient conditions (24). The formulations were also found to be visually stable. All the systems that we used had the α-T in solution either in the alcoholic phase (gels) or in the oily phase (emulsions, solution). Emulsion 2, which was a microemulsion system containing IPM, showed the highest total permeation of α-T. This was followed by IPM solution. There have been reports of IPM being a possible penetration enhancer. That could have been one of the possible reasons for better skin delivery by formulations containing IPM. However, we do not have sufficient evidence at this point to make a definite conclusion. The highest permeability of felodipine from benzyl alcohol microemulsions was found for that system that gave the highest solubility of the active both in microemulsion and in the apparent external phase (28). The IPM-containing microemulsion performed better than the benzyl alcohol-containing microemulsion.

Based on the results of the α-T permeation study, we chose gel 3, an IPM solution, and the emulsion formulation as prototype formulations for the concentration dependency study. The formulation difficulties experienced in making microemulsions precluded their incorporation in the study.

To study the effect of concentration, four times lower and higher amounts of α-T than the normally used 1% were formulated. The 1% formulations were also tested again in this study. A detectable amount of the active was found even at 0.25% concentration as early as four hours in the receptor. The skin profiles exhibited linearity and consequent dose proportionality. As the concentration of α-T was increased in the formulations from 0.25% to 4%, proportionally greater amounts of active permeated into the skin.

In addition to the numerous benefits that have been reported with α-T administration, the direct sunscreening effect of α-T is now being added to the growing list (29). Sunscreens used along with antioxidants have been found to give an added benefit of photoprotection. We used three prototypic formulations including gel 3, emulsion 1, emulsion 3, and two different sunscreens, OMC and octyl salicylate. Also, some sunscreens have been used as penetration enhancers themselves. Padimate O was shown to improve percutaneous absorption of testosterone and estradiol in swine. Padimate O was shown to lower the transition temperature of the stratum corneum lipids, which was postulated to result in significant increases in drug diffusivity across the skin (17,18). These authors also used novel topical spray vehicles containing Padimate O and octyl

salicylate to study the transdermal delivery of sex hormones. Octyl salicylate gave the highest enhancement ratio, and other penetration enhancers like oleic acid and lauro-capram gave slightly lower ratios, albeit better than the control.

We did not find oleic acid to significantly enhance the permeation of α-T. A major reason for this difference could be the lipophilicty of the α-T molecule. α-T is a lipophilic compound (octanol/water partition coefficient = 480), log (P) = 2.68. A log (P) > 2 is a good indicator of a highly lipophilic molecule. When 1-menthol along with ethanol was used as a penetration enhancer for benzoic acid and its 4-alkyl substituents, Kitagawa and Li (16) found increased permeability for benzoic acid but decreased values for the higher alkyl substituents. The addition of alkyl groups made the parent molecule more lipophilic. Further analysis showed that addition of enhancers made the skin relatively more hydrophilic compared to the vehicle, which induced an increase in the permeability coefficient of benzoic acid and decreases in those of its lipophilic substituents. The effect of penetration enhancers on the permeation of β-blockers was studied (30). The authors found azone to be a better enhancer than oleic acid. Their effects were found to be more pronounced with hydrophilic drugs than with lipophilic β-blockers. The increment of lipid fluidity and the resulting enhanced water permeability is thought to be the reason for this phenomenon. Possibly in our study the high lipophilicity of α-T was responsible for the lack of effect of oleic acid.

Pig skin is a suitable model for carrying out *in vitro* permeation studies of α-T and other antioxidants. However, we noticed that although studies carried out on the same piece of pig skin are comparable when pig skin was changed, there is a possibility of a variation in values, though the general trend tends to remain the same. Thus pig skin serves as a good indicator of the effect that may be obtained with different formulations, but further *in vivo* studies are required to arrive at definite conclusions.

In summary, we have shown that topical application of α-T significantly increased its concentration in skin and receptor fluid. High amounts of the active permeated in the receptor from a hydroalcoholic gel. Dose proportionality was observed when different concentrations of α-T (up to 4%) were applied to the skin. We have shown that micro-Yucatan pig skin is a suitable model to study the effect of formulation factors on the permeation of actives. There are differences obtained between each piece of skin with respect to permeation. However, the general trend observed with the formulations is maintained. An IPM-containing microemulsion served as an effective delivery vehicle for α-T. Oleic acid did not significantly enhance the permeation of α-T, probably due to the mechanism of action of oleic acid and the lipophilicity of α-T.

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