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MICROEMULSION FOR INTRADERMAL DELIVERY OF CETYL ALCOHOL AND OCTYL DIMETHYL PABA

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ABSTRACT

A water-in-oil microemulsion was prepared to deliver cetyl alcohol [I] and octyl dimethyl PABA or Padimate-O [II] in vitro using human and hairless mouse skin. A standard Franz diffusion cell and a microsectioning cryostat microtome were used to quantify the rate and the depth of penetration and the results were compared in percent dose penetrated for this microemulsion and two macroemulsion formulations, namely a cream and

a lotion. It appeared that the microemulsion had the ability to deliver [I] into the skin 2-6 times faster and at least twice as much as that with the other two formulations. Furthermore, the absorption of [I] from the cream or lotion product could be enhanced by as much as 50-250% if the skin had been pretreated with the microemulsion prior to product application. The advantage of using a microemulsion to achieve deeper and faster penetration of the permeating compounds was clearly demonstrated in this study.

INTRODUCTION

Microemulsions are transparent, fine deispersion system in which two or more immiscible phases are held together in suspension having an average particle size smaller than 0.15 microns in diameter (1). Such emulsion systems can exist only when interfacial energies between the dispersed and dispersing phases approach zero. As such, the resulting emulsion products are thermodynamically stable as compared to the conventional "macro" emulsions, such as creams or lotions. Furthermore, when such "micro" emulsions are in

contact with lipids and water, simultaneous emulsification ensues so long as the interfacial energies remain zero. Because of this unique emulsifying property, along with its thermodynamic stability, microemulsion has become an important technology breakthrough for the oil and detergent industries.

To date, only few pharmaceutical products are known to be based on this new emulsion technology. In 1975, a steroid microemulsion made its debut for use in humans as an anesthesic (2). Then, there were fluorocarbons incorporated into microemulsions for use as a blood susbtitute (3). Sublingual and topical uses of microemulsion products for antihypertensive (4) and antiinflammatory treatments (5) soon began to surface in the literature over the past ten years. Bhargava et. al. (6) recently gave this new emulsion a better perspective in the design of a novel drug delivery system. Dermatological products were also formulated as microemulsions and investigated in laboratory animals (7,11). The overall results seem promising as the new emulsion system apparently has the ability to deliver larger payloads of

topically applied agents into the skin, while the other vehicles may fail to do so. For example, percutaneous absorption of vitamin E by Sprague Dawley rat skin was greatly enhanced by use of either an o/w or a w/o microemulsion preparation (7). The vitamin was delivered predominantly to the epidermis, avoiding undue accumulation of this vitamin in the organs other than the skin. On the contrary, a cream and a lotion product which contained the same amount of vitamin had resulted in excessive accumulation of the vitamin in the organs such as the liver, body fats and muscle.

Many dermatological products containing local antiinflammatory or analgesic agents may require a good skin reservoir effect of the therapeutic agents to attain their desirable clinical effects. By analogy, a sun protection agent (a sunscreen) can be "long acting", provided that there has been a substantial tenacity of the UV-absorbing agent in the upper epidermis of the skin. Therefore, a topical formulation which is capable of producing a good skin reservoir for the sunscreen is highly desirable from the sustantivity standpoint. In this study a popular sunscreen agent, octyl

dimethyl PABA or Padimate-O, [II], and a skin moisturizing agent, cetyl alcohol, [I], were used as model molecules to study the permeation characteristics of a microemulsion delivery system. The choice of cetyl alcohol was based on the fact that it is not only a gelling agent, but also a skin emollient widely used in a large variety of skin care products. A water-in-oil microemulsion, a cream and a lotion formulations were employed to deliver the alcohol and the sunscreen. The delivery efficiencies were compared in rate and depth of product penetrtion into human or hairless mouse skin. The main objective of this study is to demonstrate the advantage of using a microemulsion vehicle to achieve better skin tenacities of compounds that are intended for local skin care or remedies.

MATERIALS AND METHODS

Skin Preparation

Human cadaver skin was obtained from the abdominal surface of a 50 year-old male. Hiarless mouse skin isolated from the dorsal surface of 4 month-old mice (HRS-1, Jackson strain) was also

used in this study. The human skin was sectioned and trimmed to a size of about 4 cm^2 and about 800 microns thick using a dermatome (Padgett Dermatome, Kansas City Assemblage Co., Kansas, MO). The procedure used to prepare full-thickness mouse skin was described in detail in the earlier publication (8,9). Hairless mouse skin obtained this way were similar in size and thickness to that of the human cadaver skin.

Product Preparation

Carbon-14 labelled cetyl alcohol, or C14-[I], and carbon-14 labelled octyl dimethyl PABA, or C14-[II], were both obtained from the same supplier (Amersham Corporation, Arlington, IL) with purities greater than 95% and used as received without further purification. The micro-emulsion formulation was prepared by, first, dissolving a mixture of untagged- and C14-[I] together in a silicone fluid (Dow Corning, Midland, MI), then mixing at room temperature with water and polysorbate emulsifiers at 2:1:1 ratios. A clear water-in-oil microemulsion formed almost simultaneously after the mixing. The cream and

lotion products were prepared by dissolving untagged- and C14-[I] together in a carbowax oil phase, followed by high-speed homogenization with the water and polysorbate emulsifier phases in appropriate ratio at 60-70 ^{o}C. The specific radioactivities of cetyl alcohol found in each of the three formulations were determined by radiochemical assay as $0.97x10^{6}$, $2.63x10^{6}$, and $3.73x10^{6}$ dpm per mg for the microemulsion, cream and lotion, respectively. Although an equal amount of the radiotagged [I] had been added to the three emulsions, the specific activities of [I] were different as these three formulae contained different amounts of untagged [I] (i.e., 0.6% for the microemulsion, 2.4% for the cream, and 1.0% for the lotion). Another three similar products containing a mixture of untagged- and C14-[II], were also prepared in a similar method to that used for cetyl alcohol except that the total content of the tagged- and untagged-[II] was identical for all three formulae, i.e., all formulations contained 3.0% w/w of octyl dimethyl PABA. The specific activity was determined by radiochemical assay as $9.0x10^{6}$ dpm per mg.

In Vitro Diffusion

A set of Franz diffusion cells was used to study the in vitro skin permeation rate of cetyl alcohol and octyl dimethyl PABA with these formu- the two permeation periods (one and four hours), the skin were removed from the diffusion cells, rinsed with water then with 95% ethanol for few seconds. The samples were then frozen immediately with dry ice for the following microsectioning process. The skin was mounted in a cryostat microtome (Model 2250, LKB Instruments, Inc., Gaithersburg, MD) and supported by a frozen, level block of 4% aqueous carboxyl methylcellulous (CMC) (Sigma Chemical Company, St. Louis, MO). Utilizing a small quantity of the CMC, the dermal side of the skin specimen was brushed on to the frozen CMC block. As such, the skin was securely mounted onto the CMC block with the epidermis up facing the blade. The mounting was then completed by sur- rounding the skin with the same CMC solution, followed by complete freezing at $-25\,^{o}C$. This frozen CMC block was then pared with the microtome precisely to the skin surface. The microsectioned samples were serially collected in scintillation

vials. The initial 40-micron sections (usually 4 signify possible skin damage if a sudden increase in receptor's radioactivity was noted during the permeation process. When this occurred, the experiments were repeated to assure data reliability. The total radioactivities of the permeants found in the skin were determined by radiochemical assaying of the skin specimens. The specimens were dissolved in 2 ml of a tissue solubilizer (Soluene 350, Packard Instruments Company, Downers Grove, IL) overnight. Fifteen-milliliters of Dimilume (Packard Instruments, Inc., Fullerton, CA) was added as a scintillation cocktail to carry out the radiochemical assay. These experiments were repeated three times for each of the three products. At the end of each permeation periods, the radioactivities of [I] in the receptor solution were also determined to confirm the integrity of the membrane. Since the amounts of penetrants found in the receptor solution were not only minimal in four hours, but also inconsequential to the reservoir effect of the products, these data were not reported here. The permeation of cetyl alcohol in the human cadaver

skin was likewise studied for the same permeation periods.

In a separate study, 0.1 g of the microemulsion was used to pretreat the skin. The microemulsion was left on the skin for 10 minutes. The effect of this short-term microemulsion treatment on the absorption of cetyl alcohol, which was delivered with subsequent applications of the cream or lotion over the pretreated skin, was examined for one and four hours permeation times. The permeation results were compared with those without such pretreatment. These permeation experiments were carried out side-by-side and in tripilcate.

Skin Microsectioning

In order to measure the depth of penetration of cetyl alcohol and octyl dimethyl PABA, skin was exposed to the formulations in a similar manner to that described in the rate study. At the end of the two permeation periods (one and four hours), the skin were removed from the diffusion cells, rinsed with water then with 95% ethanol for few seconds. The samples were then frozen immediately

with dry ice for the following microsectioning process. The skin was mounted in a cryostat microtome (Model 2250, LKB Instruments, Inc., Gaithersburg, MD) and supported by a frozen, level block of 4% aqueous carboxyl methylcellulous (CMC) (Sigma Chemical Company, St. Louis, MO). Utilizing a small quantity of the CMC, the dermal side of the skin specimen was brushed on to the frozen CMC block. As such, the skin was securely mounted onto the CMC block with the epidermis up facing the blade. The mounting was then completed by surrounding the skin with the same CMC solution, followed by complete freezing at -25 °C. This frozen CMC block was then pared with the microtome precisely to the skin surface. The microsectioned samples were serially collected in scintillation vials. The initial 40-micron sections (usually 4 sections) containing incomplete or partial surface areas were pooled into one scintillation vial and designated [P]. Starting with the first complete section, ten 40 micron sections [1-10] were collected and placed in separate scintillation vials. Additionally, sections [11] and [12] were collected in the sunscreen study. After the last

section was sampled, the remaining portion of the skin was placed in one scintillation vial and designated [R]. These sections were then dissolved in a tissue solubilizer and quantified for the radioactivities as described previosuly. The results were expressed as percent dose penetrated to each of the skin sections. The experiments were repeated thrice using 3 pieces of skin for each of the three products.

RESULTS AND DISCUSSION

Rate of Penetration

In a mouse skin study, the rate of delivery of cetyl alcohol with the microemulsion was 0.3% dose per hour for the microemulsion and 0.05% dose per hour for the cream. In Figure 1, the microemulsion was 6-fold higher in the rate of skin uptake of cetyl alcohol compared to that with the cream. The increasing trend of the dose-versus-time plots also suggest that up to 6 hours of permeation the skin was still able to absorb the permeating bstances from the microemulsion, but to a lesser extent from the cream. Such a steeper and higher dose-time relationship was also

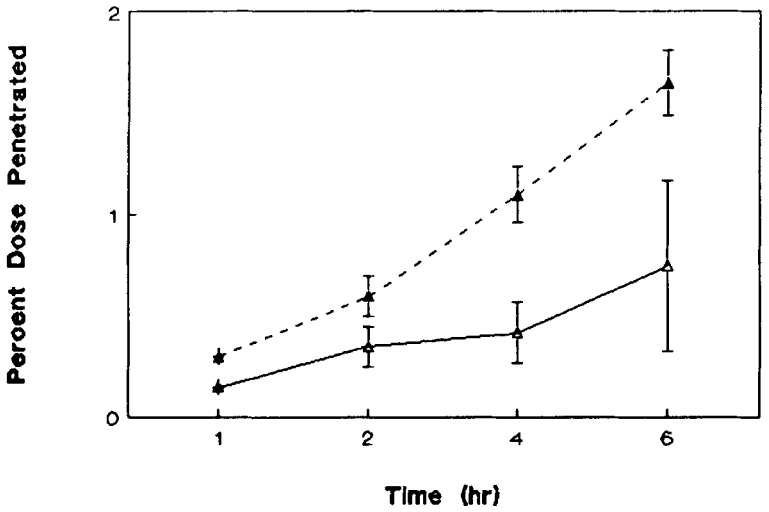

Fig. 1.
Rate of Penetration of cetyl alcohol in hairless mouse skin with the microemulsion (▲, n=3) and the cream (△,n=3)

similarly noted in the human skin study (Figure 2). The penetration rate of cetyl alcohol into the human cadaver skin with the three emulsions were estimated from the slopes of the dose-versus-time plots as 0.055%, 0.025%, and 0.010% dose per hour, for the microemulsion, cream and lotion respectively. The difference between these two sets of skin permeation data is the magnitude of penetration which may be explained by the fact of hairless mouse skin being more lipophilic than human skin (10).

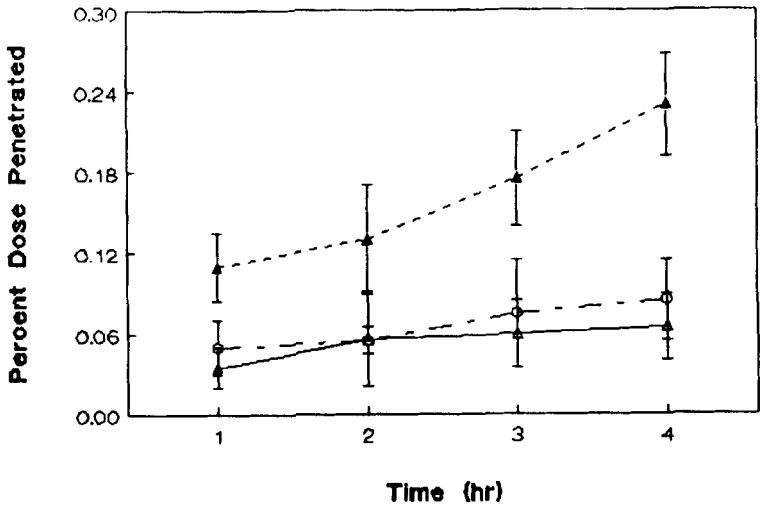

Fig. 2.
Rate of penetration of cetyl alcohol in human skin with the microemulsion (▲,n=3), the cream (△, n=3) and the lotion (○,n=3).

Enhanced Skin Absorption

The enhanced absorption of cetyl alcohol from the cream and the lotion products after 10 minutes of microemulsion pretreatment has been summarized in Table I. Pretreating the human skin with the microemulsion increased the absorption of cetyl alcohol by 50-250% as compared to that without the pretreatment. It is interesting to note that even with only 10 minutes of a such pretreatment, the barrier property of the skin can be substantially

TABLE I
Penetration of Cetyl Alcohol from The Cream and Lotion with or without The Pretreatment of Skin with Microemulsion

	Average Amount of Cetyl Alcohol $x10^6$ mM ± S.D.			
	Cream After Micro-emulsion	Cream Only	Lotion After Micro-emulsion	Lotion Only
1 Hour (n=3)	11.17 ±0.98	2.73 ±1.34	1.94 ±0.68	1.31 ±0.03
2 Hour (n=3)	10.31 ±3.37	3.66 ±1.69	1.63 ±1.06	1.99 ±0.45
3 Hour (n=3)	11.69 ±0.83	4.43 ±0.29	4.22 ±0.08	2.11 ±0.10
4 Hour (n=3)	12.45 ±4.28	5.40 ±0.92	6.25 ±1.04	4.11 ±1.02

compromised, allowing facilitated transport of the alcohol into the skin.

Depth of Penetration

The depth of skin penetration of the alcohol and the sunscreen agent in the microemulsion, cream and lotion products were evaluated using a microsectioning technique. The results indicated

TABLE II
Total Cetyl Alcohol and Octyl Dimethyl PABA Found in All Microsections of the Human Skin Permeated by the Microemulsion,Cream or Lotion Preparations

	% Octyl Dimethyl PABA ± S.D.		% Cetyl Alcohol ± S.D.	
	1 Hour (n=3)	4 Hour (n=3)	1 Hour (n=3)	4 Hour (n=3)
Micro-emulsion	0.042 ±0.005	0.076 ±0.004	0.081 ±0.013	0.205 ±0.019
Cream	0.028 ±0.010	0.048 ±0.010	0.017 ±0.002	0.069 ±0.003
Lotion	0.030 ±0.014	0.030 ±0.018	0.039 ±0.003	0.101 ±0.002

that the mean total penetration of both cetyl alcohol and octyl dimethyl PABA with the micro-emulsion was 1.5- to 5-fold more than that with the cream or lotion one hour after the permeation has commenced, and 1.5- to 3-fold more than that with the cream or lotion following four hours of permeation (Table II). The distribution of cetyl alcohol in the upper strata, especially in sections 3-7 was unequivacally more efficient with the microemuslion delivery system as compared with

the other two emulsions (Figures 3 & 4). Significant amounts of the alcohol and the sunscreen were found in the [R] sections at four hours for all three products, which might be the result of back fluxes of the permeants from the receptor solution into the dermis.

The microemulsion delivery system appeared to have the ability to lower the interfacial energy between the skin and the vehicle simultaneously upon its intimate contact with skin lipids (non-polar constituents) and water (polar constituents). As the result, a faster (rate study) penetration of the permeants occurs, followed by a facilitated penetration into the deeper strata (depth study) of the skin. A recent report from Osborne (13) indicated that there existed a synergestic behavior of microemulsion's components, instead of the microemulsion structure itself, which might be responsible for enhanced water penetration in the skin. Regardless of what factors that might have caused water to permeate faster, the dramatic improvement of the alcohol's and the sunscreen's disposition in the skin using the microemulsion was quite obvious. The effect is

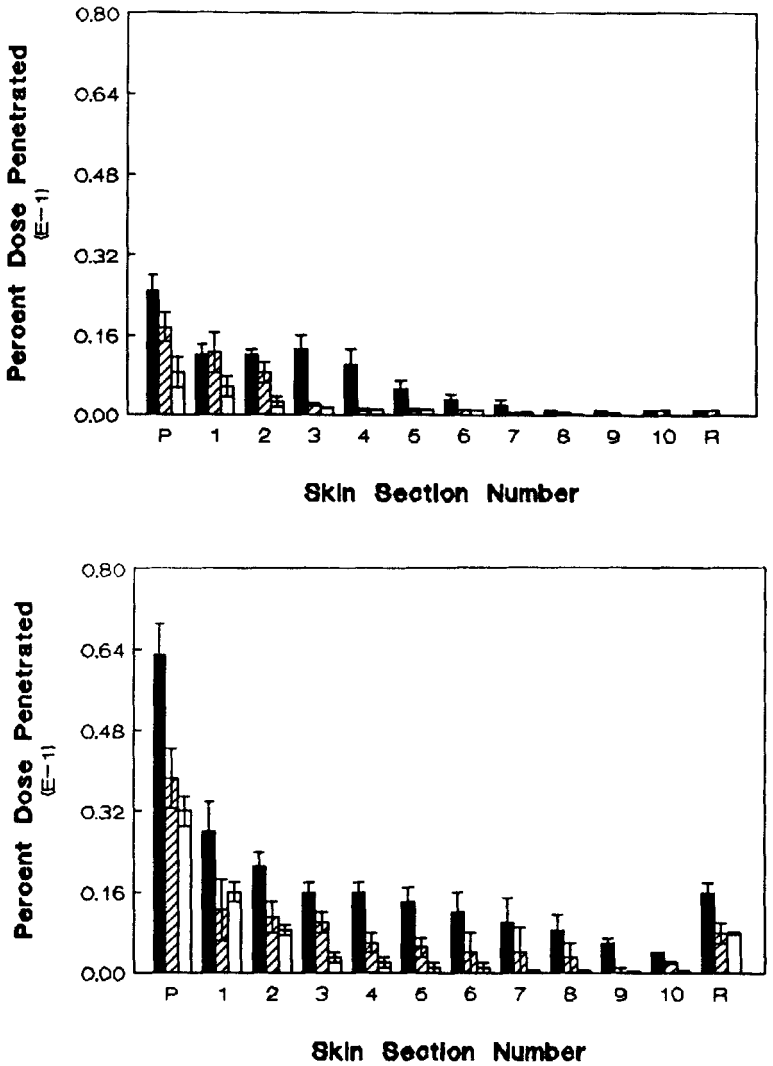

Fig. 3.
Depth of penetration of cetyl alcohol in human skin with the microemulsion (■,n=3), the lotion (▨,n=3) and cream (▭,n=3) in one hour (a) and in four hours (b).
[P]= pooled 4 partial sections with 40 microns each; [1-10]= 40 microns each; [R]= remaining tissue after the 10th section.

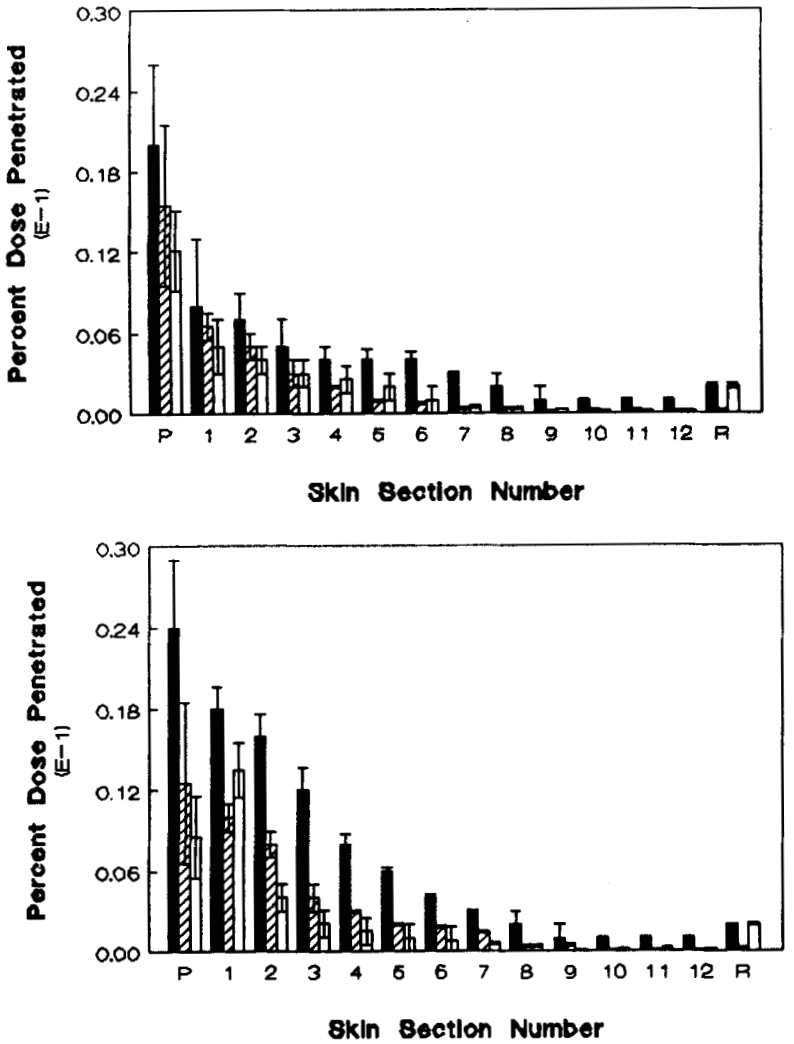

Fig. 4.
Depth of penetration of octyl dimethyl PABA in the microemulsion (■ ,n=3), the lotion (▨ ,n=3) and the cream (▭ ,n=3) in one hour (a) and four hours (b).
[P]= pooled 4 partial sections with 40 microns each; [1-10]=40 microns each; [11-12]= 200 microns each; [R]= remaining tissue after the 12th section.

mechanistically similar to that cited in a liposome study (12) where the phospholipids were reportedly fusing into the lipid domain of the stratum corneum, thereby altering the partition characteristics of the permeating steriods in the stratum corneum. It also seems evident that the the reservoir effect in the skin created by the microemulsion can further promote the penetration of the permeating substances into the deeper part of the skin.

Finally, this study has demonstrated that the penetration of cetyl alcohol is faster and more efficient with the microemulsion than with the other two conventional vehicles even though the concentration of cetyl alcohol in the microemulsion is the lowest among the three formulations. Such a unique property of the microemulsion is clinically significant since many therapeutic agents are only allowed to be used at moderate potencies yet their therapeutic actions depend on the tenacities of the agents in the skin.

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