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Formulation of topical insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET): vehicle effects on DEET in vitro skin permeation

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Abstract

The broad-spectrum topical insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET) is highly skin permeable, and serious adverse effects associated with the use of commercial DEET products have occurred in users due to extensive DEET skin permeation. In an effort to develop topical DEET formulations with reduced DEET skin permeation and extended repellency, this study investigated the effects of vehicle on DEET in vitro skin permeation from topical DEET solution and gel emulsion formulations prepared with ethanol, polyethylene glycol 400 (PEG 400), stearic acid and polyacrylic acid polymers Carbopol 940NF and Pemulen TR-2. In vitro skin permeation of DEET was evaluated in Franz diffusion cells using freshly excised abdominal cotton rat skin at 32 ± 1°C. The receiver phase samples were analyzed by HPLC with *N*,*N*-diethyl-2-phenylacetamide as reference standard. Relative to technical DEET, 40 and 50% (v/v) aqueous ethanol solutions increased DEET steady-state skin flux (J_{ss}) by 157 and 137%, respectively, while 75% (v/v) aqueous ethanol solution, neat ethanol and PEG 400 decreased the J_{ss} by 67, 74 and 59%, respectively. The incorporation of skin humectants glycerol, dimethicone or mineral oil to DEET gel emulsions based on Carbopol 940NF and stearic acid at 15% (w/w) did not significantly reduce the J_{ss}. Compared with a name-brand lotion of 7.125% (w/w) DEET, the use of 20% (w/w) PEG 400 and 1% (w/w) Tween 80, or 75% (v/v) aqueous ethanol in Carbopol 940NF and Pemulen TR-2 based formulations containing 7.5% (w/w) DEET resulted in 22.7, 18.2 and 9.1% reduction in the J_{ss}, respectively. For safety concerns, the incorporation of ethanol in commercial DEET products needs to be reevaluated. The topical formulation vehicle based on PEG 400, Carbopol 940NF and Pemulen TR-2 was effective in reducing DEET skin permeation.

Keywords: DEET; Skin permeation; Insect repellent; Ethanol; PEG 400; Carbopol 940NF; Pemulen TR-2

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1. Introduction

N,*N*-diethyl-*m*-toluamide (DEET) is a broad spectrum topical insect repellent that is effective against mosquitoes, flies, fleas, ticks and other biting insects. It has been widely used to protect humans and animals from the attack of such insects for the past 40 years (Anon, 1980; Taylor et al., 1994). In the US, it is estimated that more than 30% of the population rely on topical DEET formulations for personal protection, and over 30 million packages of DEET products are sold annually (Veltri et al., 1994). In the military, the application of topical DEET formulations has been a practical means for interrupting the transmission of vector-borne diseases and allowed troops to perform with adequate fighting strength in disease-endemic areas (Hooper and Wirtz, 1983; Fai and Lee, 1996).

Although the general safety of DEET has been established by long term human experience and rigorous animal studies, both systemic and local adverse effects associated with DEET percutaneous absorption have occurred in the users of DEET products, especially in infants and young children. In recent years, more cases of death, toxic encephalopathy, acute manic psychosis, seizure, and cardiovascular and dermal toxicity were reported (Pronczuk and Fogel, 1983; Robins and Cherniack, 1986; Veltri et al., 1994; Osimitz and Grothaus, 1995; Wingerchuck, 1995). In humans, DEET transdermal bioavailability was found as high as 16.7% (Feldmann and Maibach, 1970). In spite of the known drawbacks, DEET is still the most common insect repellent on the market due to its effectiveness and low cost.

Commercial DEET formulations include sprays, lotions, sticks, gels, soaps, and impregnated towelettes (Anon, 1980). Similar to other dermatological preparations, DEET products are over-the-counter products, and the formulation has been primarily focused on ease of application and cosmetic acceptance. To achieve extended repellency, DEET usually has been formulated at high concentrations, up to 100% in some commercial products (Sadik, 1990). In recent years, sustained-release DEET lipospheres, microcapsules and microparticles have been developed and evaluated for long term protection (Gupta and Rutledge, 1989; Domb et al., 1995), and in vitro skin permeation techniques using human and animal skins were employed to study DEET skin penetration characteristics (Reifenrath and Robinson, 1982; Reifenrath et al., 1989; Stinecipher and Shah, 1997). In our laboratory, efforts have been devoted to the development of new DEET formulations that would be safe and pleasant to use while capable of providing extended protection against biting insects by using new polymers and novel formulation techniques. To pinpoint vehicle effects on DEET skin permeation, this study evaluated the in vitro skin permeation of DEET through freshly excised abdominal cotton rat skin from solution and cream vehicles. The vehicles consisted of ethanol and polyethylene glycol 400 (PEG 400), polyacrylic acid polymers Carbopol 940NF and Pemulen TR-2, and skin humectants glycerol, mineral oil or dimethicone. Based on the findings, prototype insect repellent hydrogel emulsions showing reduced in vitro DEET skin permeation were prepared and further evaluated for mosquito repellency and DEET transdermal bioavailability.

2. Materials and methods

2.1. Materials

Technical DEET with purity greater than 98.5% (TD), polyethylene glycol (PEG 400), butylated hydroxytoluene (BHT), heavy mineral oil, triethanolamine (TEA) and methylparaben (MPB) were obtained from Sigma (St. Louis, MO). Stearic acid, glycerol and ethylenediaminetetraacetic acid (EDTA) were obtained from Fisher (Fair Lawn, NJ). Carbopol® 940NF and the polymeric emulsifier Pemulen® TR-2 were received as gifts from B.F. Goodrich (Cleveland, OH). Absolute ethanol was obtained from McCormick Distilling Company (Weston, NJ). Tetra-(2-hydroxylpropyl)-ethylenediamine (Neutrol® TE) and Tween 80 were obtained from BASF (Parsippany, NJ) and Atlas (Wilmington, DE), respectively. Ammonium acetate and *N*,*N*-diethyl-2-phenylacetamide were obtained from

Table 1
Carbopol 940 and stearic acid based gel emulsions of 10% (w/w) DEET

Composition (%, w/w)	CS-1	CS-2	CS-1-G	CS-2-G	CS-1-D	CS-2-D	CS-1-M	CS-2-M
DEET	10	10	10	10	10	10	10	10
Stearic acid	2	2	2	2	2	2	2	2
Glycerol	—	—	15	15	—	—	—	—
Dimethicone	—	—	—	—	15	15	—	—
Mineral oil	—	—	—	—	—	—	15	15
0.5% Aqueous Carbopol 940NF	88	—	73	—	73	—	73	—
1.0% Aqueous Carbopol 940NF	—	88	—	73	—	73	—	73
50% TEA	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Aldrich (Milwaukee, WI). Commercial DEET formulation OFF!® Skintastic II™ Insect Repellent (CF), a hydrophilic lotion containing 7.125% DEET (S.C. Johnson and Son, Racine, WI) was used as reference in the evaluation of new DEET formulations.

2.2. Ethanol and PEG 400 DEET solutions

Topical insect repellent formulations are often applied on the entire exposed body surface area that is susceptible to insect attacks. Used as a skin repellent and a cloth repellent as well, DEET has been widely formulated as sprays in ethanol vehicles for convenient application. In addition to providing adequate DEET concentrations for desired repelling efficacy (Anon, 1985), ethanol also possesses the merits of low viscosity and low incidence of topical reaction such as contact dermatitis (Drevets and Seebohm, 1961). It is important to study its effects on the skin permeation of DEET, a compound with high skin permeability, since ethanol has been shown to be a potent skin permeation enhancer for many drug compounds (Yum et al., 1994).

To investigate the effects of ethanol on DEET skin permeation, a series of 12% (w/v) DEET solutions were prepared in ethanol solutions. Appropriate amounts of TD were dissolved in 40% (E-40), 50% (E-50), 75% (E-75) aqueous ethanol solutions and neat ethanol (E-100). A PEG 400 solution of 12% (w/v) DEET (P-100) was also prepared to investigate whether the cosolvent significantly alters the skin permeation profile of DEET. At ambient temperature, DEET was miscible with the ethanol solutions and PEG 400 liquid at 12% (w/v).

2.3. Carbopol 940NF and stearic acid based DEET gel emulsions

Glycerol, mineral oil and dimethicone are widely formulated in skin care products as humectants to increase the skin water content. To examine their potential influences on the skin permeation of DEET, these excipients were individually formulated at 15% (w/w) in Carbopol 940NF and stearic acid based gel emulsions of 10% (w/w) DEET. In addition to 2% (w/w) stearic acid, the gels also contained Carbopol 940NF either at 0.37% (CS-1, CS-1-G, CS-1-D and CS-1-M) or at 0.74% (CS-2, CS-2-G, CS-2-D and CS-2-M). Table 1 gives the composition of these gel emulsions.

For a batch of 100 g, appropriate amounts of TD, stearic acid and a humectant were mixed in a 150-ml beaker (Phase A) and heated in a water bath at 75°C. The formula amount of Carbopol 940NF solution (Phase B) was heated in the same water bath in a separate beaker. After the solid components melted, and the temperature of the mixtures reached 75°C, Phase A was slowly added to Phase B to yield a crude gel emulsion (Phase AB) while Phase B was agitated with a specialty mixer driven by a stirrer (Arrow 1750 Stirrer, Arrow, Hillside, NJ) at 450 rpm. The crude gel emulsion was removed from the water bath, and its pH was adjusted to seven with dropwise addition of a 50% (w/w) TEA aqueous solution during the mixing. When the temperature decreased to

50°C, Phase AB was subjected to more vigorous agitation at 900 rpm for 10 min before a homogeneous white gel emulsion was obtained.

2.4. Carbopol 940NF and Pemulen TR-2 based DEET gel emulsions containing PEG 400

To examine whether PEG 400 and ethanol exert significant influences on DEET skin permeation from gel emulsions, the two liquids were incorporated in DEET hydrophillic gel emulsions. As detailed in Table 2, the PEG 400 lotions (CPP-1 and CPP-2) contained 20% (w/w) PEG 400. The gel emulsions were based on Carbopol 940NF and Pemulen TR-2, a polyacrylic acid polymeric emulsifier.

For a batch of 100, 0.5 g of Pemulen TR-2 and 1 g of Tween 80 (for CPP-1 only) were dissolved in 7.5 g of DEET in a 150-ml beaker (Phase A); 20 g of PEG 400 was diluted with 40 g (41 g for CPP-2) of deionized water (Phase B); appropriate quantities of MPB, EDTA, BHT were dissolved in an appropriate amount of a 1% (w/v) aqueous Carbopol 940NF solution (Phase C). Phase A was added to Phase B slowly (Phase AB) while the mixture was stirred with a specialty mixer rotating at 800 rpm. While stirred at the same speed, Phase AB was then mixed with Phase C slowly to yield a crude gel emulsion (Phase ABC). The pH of the crude gel emulsion was adjusted to 5.5 with dropwise addition of a 50% (w/v) aqueous TEA solution. The crude gel emulsion had been vigorously agitated at 1500 rpm for 10 min before a homogenous and smooth white gel emulsion was formed.

Table 2
Carbopol 940 and Pemulen TR-2 based gel emulsions of 7.5% (w/w) DEET containing PEG 400 or 75% ethanol

Composition (%, w/w)	CPP-1	CPP-2	CPE
DEET	7.5	7.5	7.5
PEG 400	20	20	—
1% (w/v) Aqueous Carbopol 940NF	31	31	—
Carbopol 940NF	—	—	1.5
Pemulen TR-2	0.5	0.5	0.5
Deionized water	40	41	—
75% (v/v) Ethanol	—	—	—
Tween 80	1	—	1
MPB	0.03	0.03	0.03
EDTA	0.05	0.05	0.05
BHT	0.05	0.05	0.05
50% (w/w) Aqueous TEA	q.s.	q.s.	89.5
50% (w/w) Aqueous Neutrol TE	—	—	q.s.

2.5. Carbopol 940NF and Pemulen TR-2 based DEET gel emulsion containing ethanol

The PEG 400 solution in CPP-1 was replaced with aqueous ethanol solutions (75, 85 and 95%, v/v) to yield a series of DEET ethanolic gel emulsions based on Carbopol 940NF and Pemulen TR-2. However, the emulsions obtained became unstable due to phase separation when the ethanol concentration exceeded 75% (v/v). Only CPE whose composition is listed in Table 2 was found to be a stable ethanol gel emulsion.

The preparation procedure for CPE was similar to that of formulations CPP-1 and CPP-2. For a batch of 100, 0.5 g of Pemulen TR-2 and 1 g of Tween 80 were dissolved in 7.5 g of DEET (Phase A); 1.5 g of Carbopol 940NF and appropriate amounts of MPB, EDTA, BHT were dissolved in 89 g of 75% (v/v) ethanol (Phase B) and kept overnight. Phase A was added to Phase B slowly to yield a crude emulsion (Phase AB) while Phase B was stirred with a specialty mixer rotating at 800 rpm The crude emulsion Phase AB was neutralized with a 50% (w/w) aqueous Neutrol TE solution. Further stirring at 1500 rpm for 10 min was applied to obtain a homogenous ethanolic gel emulsion.

2.6. In vitro skin permeation of DEET

In vitro skin permeation of DEET from the formulations was studied using freshly excised abdominal cotton rat skin and Franz diffusion cells (Bronaugh, 1993). The volume of the receiver compartment was 6.0 ml, and the effective diffusion area was 2.0 cm^2. The donor phase was exposed to ambient temperature while the receiver phase was maintained at 32 ± 1°C with the use of a thermostat-controlled water circulation. The receiver phase was deionized water. A star head magnetic stirrer was added into the receiver compartment to provide agitation.

Cotton rats four-week of age and weighing 40–50 g were sacrificed in a carbon dioxide chamber. The hair was removed carefully using an electric hair clipper, and the whole skin was excised by cutting along the ventral surface. After removal of the subcutaneous fat and examination for damage, two–three pieces of skin sample were trimmed from the dorsal portion with the size and shape delimited by the donor compartment. The skin sample was mounted between the receiver and donor compartments with an O-ring and a secure clamp. An appropriate amount of a DEET formulation equivalent to 0.15 g DEET was introduced into the donor compartment after the skin was allowed to equilibrate with the receiver phase for 30 min. When necessary, a spatula was used to spread the DEET materials uniformly over the skin and eliminate voids in the donor phase. The donor compartment was sealed with parafilm (American National Can™, Greenwich, CT). At 0.5, 2, 4, 6, 8 and 10 h after the loading of a DEET formulation, 100 μl of the receiver phase was sampled using a microsyringe and replenished. The samples were diluted properly with water, spiked with *N*,*N*-diethyl-2-phenylacetamide as reference standard, and analyzed by HPLC.

2.7. Chromatographic quantitation of DEET

An HPLC system consisting of a Beckman solvent delivery module (Model 112, Beckman, Fullerton, CA), a Rheodyne single-loading injector (Model 7125, Rheodyne, Cotati, CA) with a 50 μl sample loop, a Perisorb RP-18 guard column (P.J. Cobert, St. Louis, MO), a Microsorb-MV reversed-phase C8 column (150 × 3.9 mm i.d., Rainin, Woburn, MA), a variable wavelength UV-detector (Spectro Monitor III Model 1204A, Laboratory Data Control, Riviera Beach, FL) and an integrator (HP3396B, Hewlett Packard, Wilmington, DE) was employed to analyze the receptor phase samples. The mobile phase was pH 4.5 ammonium acetate buffer (0.03 M) containing 50% (v/v) acetonitrile and was delivered at 1.0 ml/min. The eluent was monitored by UV-detection at 230 nm. No interference was observed for DEET peak at this chromatographic condition.

2.8. Stability of a Carbopol 940NF and Pemulen TR-2 based DEET gel emulsion

An accelerated stability test was performed to evaluate the chemical and physical stability of DEET gel emusion CPP-1 that exhibited least DEET skin permeation among the new formulations evaluated for DEET skin permeation. Parameters such as formulation appearance, DEET content, particle size and bulk viscosity were monitored every 30 days for 120 days. The samples were stored in capped dark brown vials which were placed in a covered water bath thermostatted at 45 ± 2°C.

DEET content was analyzed by HPLC with *N*,*N*-diethyl-2-phenylacetamide as reference standard. Approximately 0.2–0.3 g of a gel emulsion sample was dissolved in 10 ml of a 0.4 M acetic acid solution containing 50% acetonitrile. An appropriate amount of the resultant solution was diluted properly with water, spiked with the internal standard, and injected onto the column for DEET quantitation after centrifugation. Particle size was determined by microscopic analysis. A small amount of a gel emusion sample was applied onto a slide and spread over gently by the edge of a cover glass. The cover glass was placed on the slide surface without being excessively pressured. The reading was done by moving the slide in one single direction. All the particles in a grid view area were analyzed. Two hundred particles were examined for their diameters and the mean diameter obtained. The viscosity of the lotion at 25 ± 1°C was measured with a Brookfield digital viscometer (Model DV-II, Brookfield, Stoughton, MA) using a No. 7 spindle. After it was removed from the water bath, the sample vial was left at ambient environment for 3 days before the measurement was made. The rotation speed of the spindle was 20 rpm

2.9. Data analysis

Skin flux (J) was determined from Fick's first law of diffusion: $J = (\mathrm{d}M/\mathrm{d}t)/A$, where $\mathrm{d}M/\mathrm{d}t$ is the amount of DEET penetrated per unit time, and A is the effective diffusion area. Steady-state skin flux (J_{ss}) was determined from the slope of

the linear portion of a cumulative penetration-time curve, and lag time (t_{lag}) was obtained by extrapolating the linear portion of the curve to the abscissa. The permeation parameters were compared for statistical significance using Student's t-test at 5% significance level.

3. Results and discussion

3.1. Skin permeation of DEET from ethanol and PEG 400 solutions

As can be seen from Fig. 1a and b, ethanol imposed dramatically different effects on DEET skin permeation depending on its concentration. The J_{ss} value for TD was 0.46 ± 0.04 mg/cm^2 per h, while the J_{ss} values for the 40 and 50% (v/v) ethanol solution increased to 1.18 ± 0.20 and 1.09 ± 0.20 mg/cm^2 per h, respectively, corresponding to a 157 and 137% increase ($p < 0.05$) as compared with that of TD. In contrast, the J_{ss} values for the 75% (v/v) aqueous ethanol solution and neat ethanol were 0.15 ± 0.03 and 0.12 ± 0.01 mg/cm^2 per h, respectively, showing a 67 and 74% decrease ($p < 0.05$) relative to that of TD. Estimated as 1.30 ± 0.34, 1.22 ± 0.26 and 1.37 ± 0.22 h, the t_{lag} values observed for TD, E-40 and E-50 were statistically similar. Whereas, the t_{lag} values observed for E-75 and E-100 were 2.26 ± 0.17 and 2.71 ± 0.16 h, respectively, and were both significantly greater ($p < 0.05$) than that for TD. Similar to the 75% (v/v) ethanol solution and neat ethanol, PEG 400 (P-100) significantly reduced DEET skin permeation as the J_{ss} value for P-100 was 0.19 ± 0.02 mg/cm^2/h, about 59% lower ($P < 0.05$) than that for TD. The t_{lag} value for P-100, 1.51 ± 0.28 h, was similar to that of TD.

Effects of ethanol on the skin permeation of drug compounds have been previously studied using rodent and human skin (Ghanem et al., 1987; Berner et al., 1989; Pershing et al., 1990; Yum et al., 1994). It was demonstrated by Ghanem et al. (1987) that ethanol could enhance the skin flux of compounds of different lipophilicity primarily by (i) increasing the drug solubility in the donor phase; (ii) increasing skin lipid fluidity; and (iii) forming new pores in the stratum

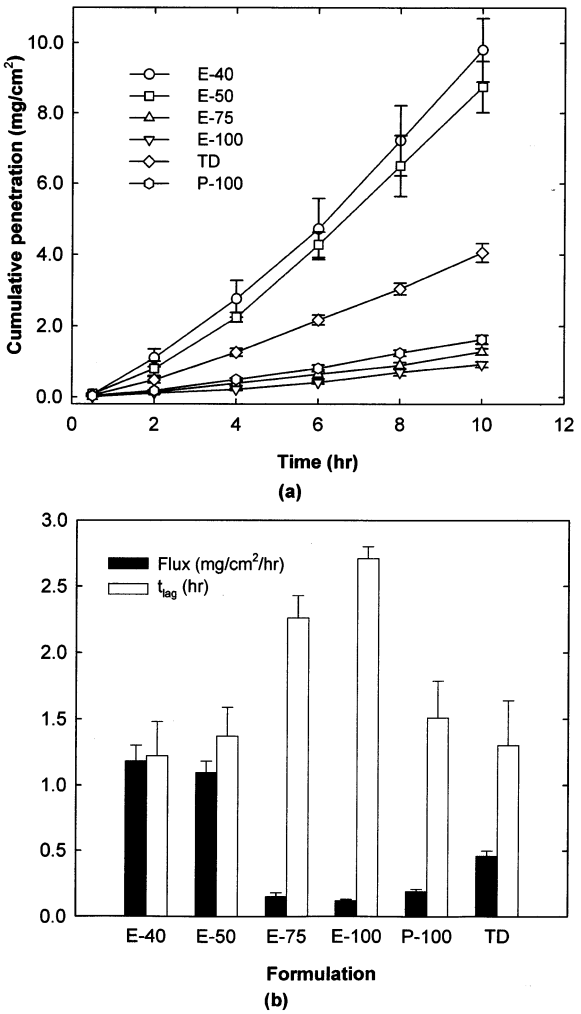

Fig. 1. Skin permeation of DEET from ethanol and PEG 400 solutions containing 12% (w/v) DEET (E-40, E-50, E-75, E-100 and P-100) as compared with technical DEET (TD): (a) Cumulative penetration–time profiles; (b) steady-state skin flux (J_{ss}) and lag time (t_{lag}).

corneum. Increased skin fluxes as a result of enhanced partition of drugs concomitant with ethanol in the stratum corneum and epidermis were also observed (Berner et al., 1989; Yum et al., 1994). In contrast, at high concentrations ($> 50\%$, v/v), ethanol decreased the skin fluxes due to its dehydrating effect on the skin (Yum et al., 1994) and reduced vehicle-to-skin drug partition (Pershing et al., 1990). The observed concentration-dependent effects of ethanol on DEET

skin permeation were in good agreement with the findings of the previous studies. The phenomenon that PEG 400 retarded DEET in vitro skin permeation was consistent with the observations of various investigators that PEG vehicles resulted in poor skin permeation of certain drug compounds though PEG cosolvents had solubilizing effects on the tested compounds. It has been generally considered that the solubilizing effects of PEG on the drug compounds restricted the drug molecules from partitioning into the stratum corneum (Kammerau et al., 1975; Davis et al., 1981; Hadgraft, 1983; Freeman et al., 1986; Sheth et al., 1986).

3.2. Skin permeation of DEET from gel emulsions containing glycerol, mineral oil or dimethicone

As shown in Fig. 2a and b, at a level of 15% (w/w), skin humectants glycerol, dimethicone or mineral oil did not alter DEET in vitro skin flux as significantly as aqueous ethanol solutions. Nevertheless, the incorporation of 15% (w/w) glycerol (CS-1-G), dimethicone (CS-1-D) or mineral oil (CS-1-M) into the DEET gel emulsion resulted in a marginal increase in the J_{ss} without statistical significance when compared with CS-1 which contained 0.37% (w/w) Carbopol 940NF and 2% (w/w) stearic acid. The use of glycerol, dimethicone or mineral oil at 15% (w/w) also significantly ($p < 0.05$) reduced the t_{lag} value by 36, 26 or 36%, respectively, from 1.70 (± 0.04) h to 1.09 (± 0.08), 1.25 (± 0.04) or 1.08 (± 0.09) h. The effects of the skin humectants on J_{ss} and t_{lag} could be in part attributed to the fact that the bulk viscosity of the formulation decreased when a skin humectant was incorporated.

Compared with CS-2 which contained 0.74% (w/w) Carbopol 940NF and 2% (w/w) stearic acid, the addition of 15% (w/w) glycerol (CS-2-G), dimethicone (CS-2-D) or mineral oil (CS-2-M) led to a marginal decrease in J_{ss} and an increase in t_{lag} without statistical significance ($p < 0.05$). Change in t_{lag} was difficult to evaluate as considerably large variation was observed for the DEET gel emulsions containing 0.74% (w/w) Carbopol 940NF. For the gel emulsions that contained 0.37% (w/w) Carbopol 940NF and 15% (w/w) glycerol, dimethicone or mineral oil (CS-1-G, CS-1-D or CS-1-M), increasing the polymer concentration to 0.74% (w/w) (CS-2-G, CS-2-D or CS-2-M) resulted in a reduction in J_{ss} by 31, 30 and 44% ($p < 0.05$), respectively. However, the lotions were too viscous and were not easily spread over any skin surface, and all of them exhibited higher mean J_{ss}, ranging from 0.23 to 0.42 mg/cm^2 per h, as compared with 0.22 mg/cm^2

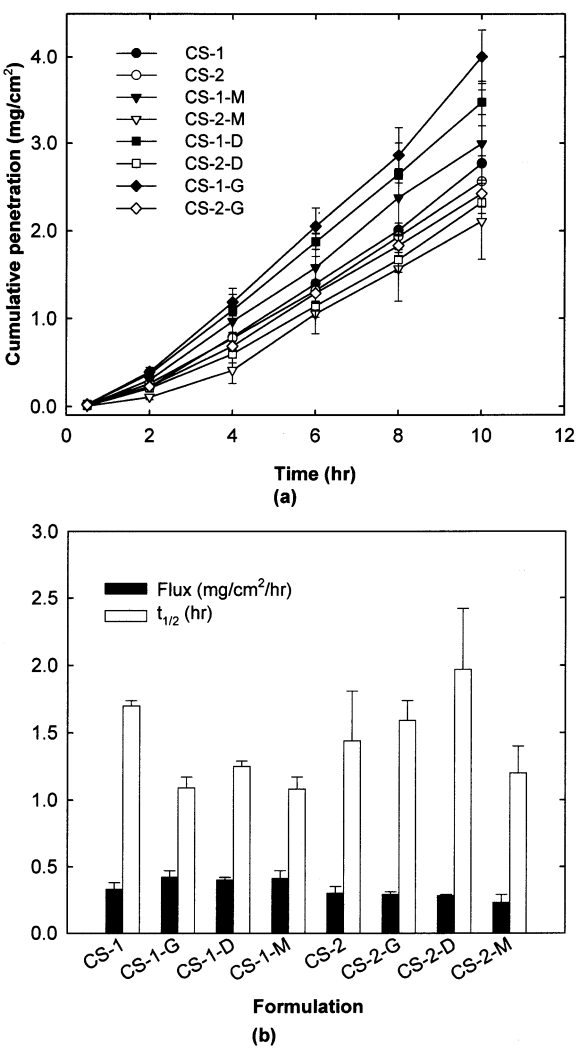

Fig. 2. Effects of skin humectants on DEET skin permeation from Carbopol 940NF and stearic acid based gel emulsions of 10% (w/w) DEET: (a) Cumulative penetration–time profiles; (b) steady-state skin flux (J_{ss}) and lag time (t_{lag}).

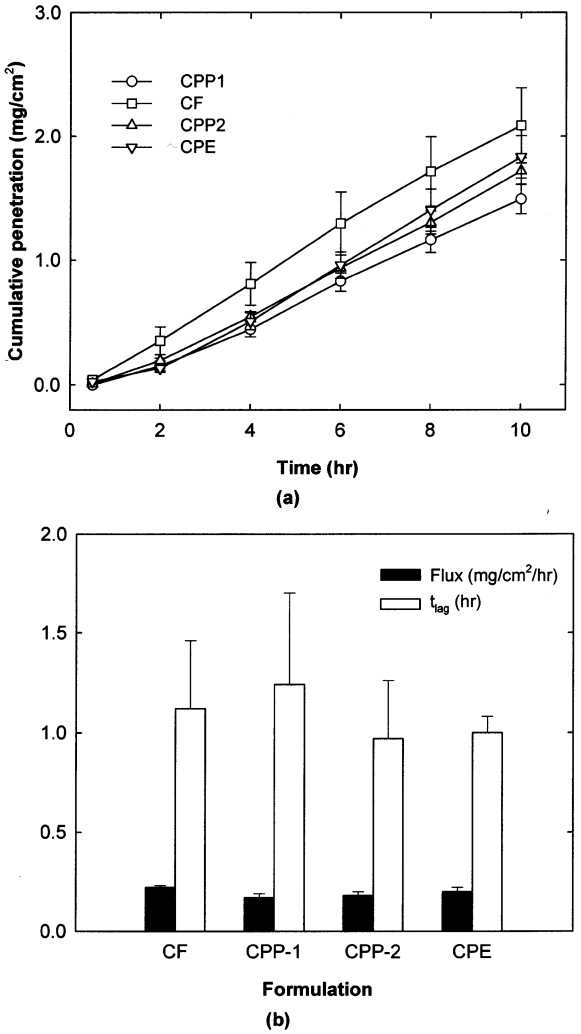

Fig. 3. Skin permeation of DEET from Carbopol 940NF and Pemulen TR-2 based gel emulsions of 7.5% (w/w) DEET containing PEG 400 and ethanol (CPP-1, CPP-2 and CPE) as compared with the commercial lotion (CF): (a) Cumulative penetration–time profiles; (b) steady-state skin flux (J_{ss}) and lag time (t_{lag}).

per h for CF (Fig. 3b). Similarly, as shown in Fig. 2b, without the use of the skin humectants, raising the concentration of Carbopol 940NF from 0.37% (w/w) (CS-1) to 0.74% (w/w) (CS-2) did not significantly decrease the DEET skin flux though the bulk viscosity of the lotion increased greatly. These preliminary results indicated that simply raising the viscosity of the gel matrix would not warrant the reduction of DEET skin permeation.

3.3. Skin permeation of DEET from Carbopol 940NF and Pemulen TR-2 based gel emulsions containing PEG 400

As shown in Fig. 3a and b, the J_{ss} observed for Carbopol 940NF and Pemulen TR-2 based gel emulsions CPP-1 (0.17 ± 0.02 mg/cm^2 per h) and CPP-2 (0.18 ± 0.02 mg/cm^2 per h) were significantly lower ($p < 0.05$) than that for CF (0.22 ± 0.01 mg/cm^2 per h). Relative to CF, CPP-1 and CPP-2 exhibited 22.7 and 18.2% reduction in J_{ss}, respectively. The J_{ss} value for ethanol-containing lotion CPE (0.20 ± 0.02 mg/cm^2 per h) was about 9.1% lower than that for CF without statistical significance. Due to the large variation, the t_{lag} values for CF, CPP-1, CPP-2 and CPE were difficult to differentiate. Nevertheless, the J_{ss} data suggested that the incorporation of PEG 400 at 20% (v/v) and ethanol at concentration of 75% (v/v) were able to decrease the DEET skin permeation from Carbopol 940NF and Pemulen TR-2 based gel emulsions.

The DEET skin permeation properties of CPP-1 was further evaluated in vivo using four beagle dogs (Qiu et al., 1997). With a single topical application to the shaved anterior dorsal region along the backline of the animals, CPP-1 demonstrated a 23.4% reduction in DEET transdermal bioavailability relative to CF. CPP-1 also offered better repellency against the yellow fever mosquito *Aedes aegypti* in a laboratory repellency test. It was shown that the reduction in DEET transdermal absorption was a result of reduced DEET skin/vehicle partition.

3.4. Stability of Carbopol 940NF and Pemulen TR-2 based formulation CPP-1

As shown in Table 3, Formulation CPP-1, which was based on Carbopol 940NF and Pemulen TR-2 and contained 20% (w/w) PEG 400, was physically and chemically stable during the four-month accelerated stability test. The formulation appearance, DEET content, particle size and bulk viscosity did not change significantly during this period at 45°C.

Table 3
Stability of the DEET gel emulsion CPP-1 at 45°C

Storage time (day)	DEET content[a] (%)	Particle size[a] (μm)	Viscosity ($\times 10^{-3}$ cps)	Appearance
0	7.36 (0.23)[b]	1.8 (0.6)	4.4	White lotion
30	7.20 (0.37)	1.9 (0.8)	4.0	White lotion
60	7.38 (0.14)	1.8 (0.7)	4.2	White lotion
90	7.28 (0.29)	1.8 (0.9)	4.4	White lotion
120	7.12 (0.17)	2.0 (0.5)	4.2	White lotion

[a]Mean, $n = 3$.
[b]S.D.

4. Conclusions

Marked vehicle effects on DEET in vitro skin permeation were observed in this study. It was concluded that the incorporation of ethanol in commercial DEET products should be strongly discouraged. Aqueous ethanol solutions up to 50% (v/v) were able to greatly enhance DEET skin permeation. Although concentrate aqueous ethanol solutions appeared to reduce the DEET skin flux in the in vitro skin permeation experiment, they could extract lipids from the stratum corneum after prolonged and/or repetitive application, leading to the formation of new pores for diffusion solutes, and thus enhance the skin permeation as demonstrated by other researchers with various compounds (Yum et al., 1994). Secondly, the incorporation of skin humectants glycerol, mineral oil or dimethicone into DEET gel emulsions did not appear to significantly lower DEET skin permeability. However, the combined use of polyethylene glycol 400 and polyacrylic polymers Carbopol 940 and Pemulen TR-2 resulted in stable formulations capable of facilitating reduced DEET skin permeation.

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