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Brief Communication

Microencapsulation decreases the skin absorption of *N*,*N*-diethyl-*m*-toluamide (DEET)

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Abstract

The insect repellent *N*,*N*-diethyl-3-methylbenzamide (DEET) is widely used and is generally regarded as safe when used according to label instructions. Yet many studies have shown it to be absorbed through the skin. The objective of this study was to determine whether the skin absorption rate of DEET could be decreased while maintaining an evaporation rate consistent with effective repellency. To this end, an aqueous suspension containing ^{14}C-DEET (15% w/w) entrapped in walled polysaccharide microcapsules was prepared and tested for skin absorption in vitro using modified Franz cells maintained in a fume hood. The control formulation was 15% w/w DEET in ethanol. Two doses (3 μL and 5 μL per 0.79 cm^2 cell) of each formulation were applied to split-thickness human cadaver skin ($n = 8$/dose), and permeation was monitored for 24 h. The microencapsulated DEET formulation lead to a 25–35% reduction of radiolabel permeation compared to the ethanolic DEET formulation. Skin levels of radioactivity at 24 h were comparable, indicating that DEET evaporation from the microencapsulated formulation was comparable to or greater than that from ethanol. Hence microencapsulation increased the ratio of DEET evaporation rate to skin penetration rate relative to unencapsulated control in this in vitro study.

Keywords: DEET; Repellent; Encapsulation; Microencapsulation; Percutaneous absorption; Skin permeation

1. Introduction

Microencapsulation has been shown to reduce the environmental impact of pesticides (Quaglia et al., 2001) and improve their toxicological profile (Shirley et al., 2001). Encapsulation of the sunscreen octyl methoxycinnamate reduced its penetration and/or accumulation in the stratum corneum (Jimenez et al., 2004; Olvera-Martinez et al., 2005). Spraying of cotton textiles with an aqueous emulsion containing microencapsulated *N*,*N*-diethyl-*m*-toluamide (DEET) led to a bio-cloth with long-lasting mosquito repellency compared to fabric treated with DEET in ethanol (Fei and Xin, 2007). It was of interest to determine whether a microencapsulated DEET formulation would lead to reduced skin penetration as seen with octyl methoxycinnamate while maintaining an acceptable evaporation rate for repellency. In order to do this the ratio of the absorption rate to the evaporation rate must be lowered with respect to a non encapsulated formulation. The present study tests this concept using an excised human skin model and radiolabeled DEET formulations.

2. Materials and methods

2.1. Chemicals

Carbonyl-[^{14}C] DEET (52 mCi/mmol) was custom synthesized by Vitrax (Placentia, CA). The radiochemical purity was stated by the manufacturer to be 99%. Unlabeled DEET and calcium-free Dulbecco's phosphate-buffered saline were purchased from Sigma–Aldrich (St. Louis,

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MO). Soluene-350® was from Perkin–Elmer Biosciences. Ethanol (95%) was from Aaper (Shelbyville, KY). Sodium carboxymethylcellulose (CMC) USP (medium viscosity) was obtained from Ruger Chemical Co., Inc. (New York, NY). Benzalkonium chloride (BZK) USP (50% ethanolic), Cetyl Esters Wax USP, and Emulsifying Wax USP were purchased from Spectrum Chemical Co. (Gardena, CA).

2.2. Dose solutions

An aqueous suspension containing 15% w/w ^{14}C-DEET (7.8 μCi/mg) in the form of permeable, walled polysaccharide microcapsules enclosing DEET and adjuvant material was prepared by an interfacial precipitation method (Speaker, 2005). Briefly, microcapsule walls are formed in two steps. First, a charged amphiphilic macromolecule (CMC) is introduced into an emulsion. The macromolecule is rapidly driven to the emulsified phase interface by polar solvent interaction forces. Subsequently, the second, wall-forming step is to introduce a second, reactive agent (BZK) to the system, precipitating the macromolecule to form a wall structure surrounding each droplet. DEET was dissolved in a waxy adjuvant base at 60 °C to form a core phase consisting of approximately 60% DEET, 30% Cetyl Esters Wax, and 10% Emulsifying Wax. This base has a specific gravity of approximately 0.92 g/cm^3 and gels below about 40 °C, and is understood to retard the diffusion and vaporization of DEET. The resulting core material was subsequently dispersed in an aqueous solution of 1% CMC at 60 °C using a magnetic stirrer turning at approximately 2000 rpm, using 2 g CMC solution to 1 g core phase. After formation of a stable oil-in-water dispersion, the microcapsule wall was formed by adding 1% aqueous BZK solution, using 2 g BZK solution to every 5 g CMC solution. The encapsulated suspension thus formed was diluted with distilled water to a final DEET concentration of 15% w/w, and cooled to ambient temperature. The control formulation was 15% w/w ^{14}C-DEET (11.1 μCi/mg) in 95% ethanol.

2.3. Microcapsule characterization

While the sample used was not specifically characterized in detail, due to the radiolabel content, other similar samples prepared using the same methods have been studied, and are understood to be representative of the labeled sample used. Typically encapsulates formed in this manner are observed to form droplets with a size distribution peaked at approximately 1–10 μm radius, with less than 1% exceeding 20 μm. Because the microcapsule walls are permeable, it is understood that an equilibrium concentration of DEET will form in the continuous phase according to the partition coefficient, at approximately 1% dissolved DEET. However, when the capsules are successfully formed, no coalescence or other appearance of emulsion failure is observed, indicating very high encapsulation efficiency, approaching quantitative trapping. If the core material is simply dispersed in either wall material alone, rather than encapsulated, the emulsion fails in minutes, forming a clear supernatant phase.

2.4. Skin permeation study

The method has been previously described (Santhanam et al., 2005). Split-thickness human cadaver skin (~300 μm) was mounted in modified Franz diffusion cells (0.79 cm^2) and placed in thermostatted aluminum blocks maintained at 37 °C in a fume hood with a partially drawn sash. The receptor solution (magnetically stirred) was Dulbecco's phosphate-buffered saline, pH 7.4, containing 0.02% sodium aside to inhibit microbial growth. The integrity of the tissue was verified using ^3H_2O and the skin samples were randomized across treatments based on the ^3H_2O permeation results (Kasting et al., 1994). After an overnight equilibration period, each formulation was applied to eight skin samples at a dose of 3 μL/cell and eight samples at a dose of 5 μL/cell. These levels correspond to DEET levels of about 0.45 mg/cm^2 for the 3 μL doses and 0.75 mg/cm^2 for the 5 μL doses. The receptor solution was removed for analysis at 1, 2, 4, 8, 12 and 24 h post-dose and replaced with fresh buffer. Following the last receptor collection, the skin samples were removed from the diffusion cells and dissolved in 2 mL of Soluene-350®. All samples were analyzed by liquid scintillation counting.

Cumulative absorption was calculated for each cell from the sum of the radioactivity levels in the receptor solutions and was expressed as percent of applied dose. The encapsulated formulation was compared to its ethanol control formulation at each dose using a Student's *t*-test (two-tailed). Differences having $p < 0.05$ were considered significant. One skin sample in the 5 μL control group had a ^3H_2O permeation rate 0f 1.72 μL/cm^2, slightly above the customary 1.56 μL/cm^2 cutoff value (Lehman et al., 2007). However, it showed average DEET permeation and was therefore retained in the analysis. The inclusion of this sample had no effect on the statistical significance of the results.

3. Results

The time course for appearance of radioactivity associated with ^{14}C-DEET in the receptor solutions is shown in Fig. 1. Table 1 shows the mass balance at the conclusion of the study. The microcapsule formulation led to reductions of 35% (3 μL) and 23% (5 μL) in the permeation of radioactivity into the receptor solution after 24 h as compared to the unencapsulated control. The residual radioactivity found in the skin after 24 h ranged from 4.3% to 14.5% of dose, with an average value of 9%. In one case the skin levels were higher for the microcapsule formulation versus control, whereas in the other case they were equal. DEET evaporation (calculated from the missing radioactivity at 24 h) was 47–49% of dose for the microcapsule formulation and 33–40% for the control. The differences in estimated evaporation between microcapsule and

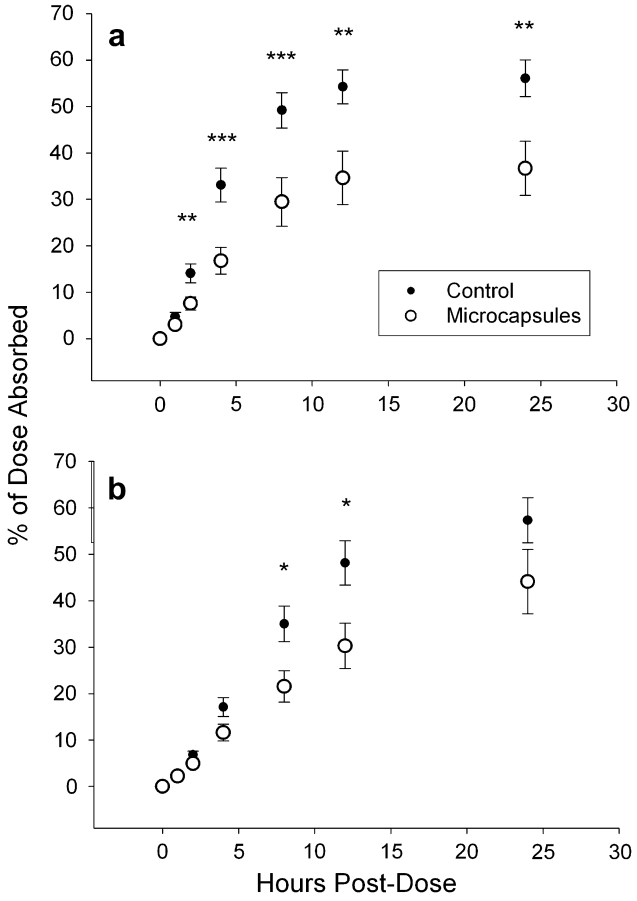

Fig. 1. Appearance of radioactivity in the receptor solution following topical application of ^{14}C-DEET formulations (15% w/w) to human skin in vitro (mean ± SE, $n = 8$). (a) 3 μL/cell; (b) 5 μL/cell. Significant differences are designated as: * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$.

Table 1
Disposition of radioactivity 24 h after topical application of ^{14}C-DEET to human skin in vitro (mean ± SE, $n = 8$)

Formulation and dose	% of applied dose		
	Receptor solution	Skin	Evaporated[a]
3 μL (0.45 mg/cm²)[b]			
Microcapsules	36.7 ± 5.8**	14.5 ± 2.4***	48.8 ± 5.1
Control[c]	56.1 ± 4.0	4.3 ± 1.5	39.6 ± 5.3
5 μL (0.75 mg/cm²)			
Microcapsules	44.1 ± 6.9	9.0 ± 1.5	46.9 ± 7.6
Control[c]	57.3 ± 4.8	9.5 ± 3.6	33.2 ± 3.3

Asterisks designate significant differences from control formulation at $^{*}p < 0.05$, $^{**}p < 0.02$; $^{***}p < 0.01$.

[a] Value reflects difference between radioactivity dosed and the sum of radioactivity collected at 24 h.

[b] Value in parentheses reflects the chemical dose of DEET.

[c] Control formulation was 15% DEET in ethanol.

control formulations were not significant, although in both instances the value for the microcapsule formulation was numerically higher. Thus it may be stated that DEET evaporation from the cells treated with the DEET microcapsule formulation was equivalent to that of the control.

4. Discussion

Passive absorption and evaporation of a compound in a deposited film are both subject to the same driving force, i.e., the gradient of the chemical potential of the diffusing ingredient (Cussler, 1997). An analytical solution to this problem as it applies to skin (Kasting and Miller, 2006) shows that, for a very thin film, the ratio of evaporation rate to absorption rate depends on a single dimensionless parameter χ,

$$\chi = \frac{hk_{\mathrm{evap}}\rho}{DC_{\mathrm{sat}}}. \quad (1)$$

In Eq. (1), h is the thickness of the stratum corneum, ρ is the density of the (liquid) permeant, D is its diffusivity in the stratum corneum and C_{sat} is its solubility in the stratum corneum. The parameter k_{evap} is an evaporative mass transfer coefficient which depends sensitively on the vapor pressure of the permeant and the air flow over the surface (Kasting and Miller, 2006). In engineering parlance, χ may be equated to a Biot number (Ray Chaudhuri, 2007).

For topical applications to skin, the theory presented in Kasting and Miller (2006) postulates the existence of a deposition layer – a relatively permeable region of the stratum corneum into which solvent-deposited solutes rapidly dissolve. The concept is linked to the desquamation process and associated loss of barrier lipids from the upper stratum corneum. If the thickness of this layer is called h_{dep}, then a fractional deposition depth f may be defined as

$$f = \frac{h_{\mathrm{dep}}}{h} \quad 0 < f < 1 \quad (2)$$

For volatile permeants, higher values of f favor absorption relative to evaporation. Application of this model to DEET (Santhanam et al., 2005) and also to benzyl alcohol (Miller et al., 2006) suggests a value $f \approx 0.1$ for these moderate sized compounds deposited onto skin from ethanol.

According to this framework, simply including a non volatile diluent (e.g., an oil or a polymeric film former) into a DEET formulation would not be expected to have much effect on the evaporation-to-absorption rate ratio. It would lower both rates proportionately by reducing the chemical potential of DEET in the residual surface film, thus prolonging both processes. The film itself would act as a deposition layer, reducing the benefit related to Eq. (2). However, if the residual film was structured in such a way that more surface area was exposed to the air than to the skin, then the evaporation-to-absorption rate ratio would be increased by maintaining or increasing k_{evap} and reducing the effective area for diffusion into the skin. We suggest that this mechanism is primarily responsible for the decreased skin absorption of DEET from the microcapsule formulation relative to ethanolic solution (Fig. 1), i.e., the microcapsules reside primarily on the skin surface and have more surface area exposed to air than to skin. It is possible that a reduction in deposition depth (cf. Eq. (2)) also plays a role; however, the geometry of such a

microcapsule film is clearly more complicated than the one-dimensional film model leading to Eqs. (1) and (2), so that quantitative comparisons with the model results should be viewed cautiously.

A simplified model of the microcapsule preparation may be considered as a monodisperse population of walled droplets of 5 μm radius, each containing approximately 500 μm^3 or 500 femtoliter volume of the core material. The formulation is 60% core material by weight, or 65% by volume. Therefore, a 3 μL aliquot of the encapsulated suspension would contain 1.95 μL of the core material, dispersed in approximately 4 million packages each containing 500 femtoliter of the core material. Thus an estimate of the skin coverage density would be roughly 20 μm^2 per microcapsule for the 3 μL aliquot, and about 12 μm^2 for the 5 μL aliquot. Since the cross sectional area of a microcapsule of radius 5 μm is about 80 μm^2, these estimates suggest that only a few layers of DEET microcapsules were present on the skin surface under the conditions of the study. From the exposed surface argument in the preceding paragraph, one would expect the evaporation-to-absorption rate ratio to increase with decreasing dose in this dose range, a prediction which is consistent with the data in Fig. 1. It seems possible that even larger ratios might be observed at lower specific doses of DEET.

DEET permeation through the skin from the control formulation (15% DEET in ethanol) in the present study was higher than that obtained previously in our laboratory (Santhanam et al., 2005) under comparable test conditions. The skin absorption of 56–57% of dose after 24 h reported here may be compared with 24 h values of 21–25% of dose for comparable DEET levels tested by (Santhanam et al., 2005) (fume hood values). A partial explanation for this difference may be found in the tissue integrity test results. The Santhanum study employed cadaver skin with a median ^3H_2O penetration rate of 0.48 μL/cm^2 (range 0.2–1.8 μL/cm^2) according to the Franz and Lehman protocol (Franz and Lehman, 1990), whereas the corresponding values for the present study were a median of 1.15 μL/cm^2 with a range of 0.7–1.7 μL/cm^2. Franz and Lehman's original guideline of 1.2 μL/cm^2 as an upper limit for acceptability of skin samples (Franz and Lehman, 1990) has been modified by the original investigators to 1.56 μL/cm^2 based on additional experience (Lehman et al., 2007). All but one of the skin samples tested in the present study met the latter criterion; however, only 59% of them (19/32) met the earlier criterion. Thus, the comparison reported here for DEET absorption reinforces the necessity to run proper controls when assessing skin absorption in vitro.

In vivo studies tend to yield lower values of DEET absorption than those reported here or by (Santhanam et al., 2005). Reasons for this have been previously discussed (Santhanam et al., 2005) and include the inclusion of a washing step in most human subjects protocols and other in vivo surface loss mechanisms including ruboff and desquamation. We nevertheless anticipate that the relative differences in skin absorption between encapsulated and unencapsulated DEET formulations reported here will translate into a measurable difference in vivo. If the repellent properties are maintained or improved (as suggested by the work of (Fei and Xin, 2007) and by the evaporation data in Table 1), then a perceivable product benefit may be obtained from microencapsulation. Furthermore, alternative microencapsulation strategies under development in our laboratories (TJS) may impart different and potentially more favorable properties to repellent formulations than the example reported here.

Conflict of interest statement

TJS is president of Capsulent, a developer of microencapsulation technology. The DEET formulation described herein is a prototype formulation from Capsulent.

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