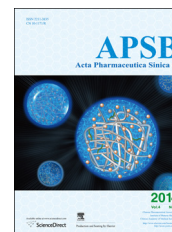




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ORIGINAL ARTICLE

# Evaluation of percutaneous permeation of repellent DEET and sunscreen oxybenzone from emulsion-based formulations in artificial membrane and human skin

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Received 10 September 2013; revised 15 October 2013; accepted 14 November 2013

## KEY WORDS

Diffusion;  
Human skin;  
Artificial membrane;  
Permeability;  
Concurrent use;  
Insect repellent;  
Sunscreen

**Abstract** Insect repellent DEET and sunscreen ingredient oxybenzone play an essential role in minimizing vector-borne diseases and skin cancers. The purpose of this study was to investigate the effects of emulsion type, addition of thickening agent and droplet size in three emulsion-based lotions on percutaneous permeation of DEET and oxybenzone using *in vitro* diffusion experiments, in order to minimize overall systemic permeation of the substances. Formulation C (water-in-oil emulsion) significantly increased overall permeation of DEET through human skin (56%) compared to Formulation A (oil-in-water emulsion). Formulation B (oil-in-water emulsion with thickening agent xanthan gum) significantly decreased the size of oil droplet containing DEET (16%), but no effect on oil droplets containing oxybenzone. Adding xanthan gum also increased overall permeation of DEET and oxybenzone (21% and 150%) when compared to Formulation A; presence of both ingredients in Formulation B further increased their permeation (36% and 23%) in comparison to its single counterparts. Overall permeation of oxybenzone through LDPE was significantly higher by 26%–628% than that through human skin; overall permeation of DEET through human skin was significantly higher by 64%–338% than that through LDPE.

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.



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## 1. Introduction

DEET (*N,N*-diethyl-*m*-toluamide) is one of the most effective broad-spectrum topical insect repellents commonly used to prevent vector-borne diseases such as West Nile virus and Lyme disease<sup>1,2</sup>. Oxybenzone (2-hydroxy-4-methoxyphenyl-phenylmethanone, OBZ) is a typical UVA/UVB sun-blocking ingredient frequently present in sunscreen lotions and many other cosmetic products<sup>3</sup>. Topical skin application of insect repellents and sunscreens has become prevalent in recent years, due to an increasing awareness of health threats from vector-borne diseases and sunlight-induced skin aging and damage.

Under ideal use conditions, active ingredients like DEET and OBZ should exert minimal percutaneous permeation and systemic disposition from topical preparations. However, both DEET and OBZ are capable of permeating across the skin and reaching general circulation upon skin application. Their permeation rate and degree across the skin have been found to be dependent upon dissolving vehicles<sup>4</sup>, preparation types or mixing methods<sup>5</sup>.

Appropriate formulation strategies may influence or modify percutaneous characteristics of an active ingredient from a particular preparation. Semisolid emulsion-based preparations have been extensively selected for active pharmaceutical components owing to their solubilizing capacity for lipophilic and hydrophilic molecules, as well as satisfactory aesthetic acceptance. By adjusting various parameters in formulating an emulsion, it is possible to alter percutaneous permeation of an active ingredient from the preparation.

Skin permeation of an active ingredient from an emulsion preparation is associated with emulsion types, *i.e.*, water-in-oil (w/o) or oil-in-water (o/w). Substances dissolved in the external continuous phase of the emulsion would possess different permeation properties than those incorporated in the internal globules of the emulsion<sup>6</sup>. Droplet size of an internal phase may also influence skin permeation of an ingredient from the emulsion<sup>7</sup>. Furthermore, adding auxiliary components in formulating stable and elegant emulsion could modify skin permeation. For example, a thickening agent within an emulsion aids in formation of a thick, uniform, and effective protection film upon skin application; the effects of thickening agents on skin permeation of several active ingredients had been indicated in previous studies<sup>8,9</sup>. In this study, we designed three emulsion-based formulations, and systematically examined the influence of emulsion

type, internal phase droplet size, and thickening agent xanthan gum on percutaneous permeation of DEET and OBZ using *in vitro* diffusion experiment. The objective of the study was to optimize a semisolid lotion that possessed minimal percutaneous permeation of the two active ingredients.

## 2. Materials and methods

### 2.1. Materials

Pure chemical DEET was purchased from Fluka Chemika GmbH (Buchs, Switzerland), and OBZ was purchased from Riedel Haer GmbH (Seelze, Germany). Some of the emulsion ingredients, Emulfree CBG (butylene glycol cocoate, ethyl cellulose, and isostearic alcohol), Labrasol (caprylocaproyl macroglycerides), Geleol (glyceryl monostearate 40–55), Isostearate isostearyl, Labrafac PG (propylene glycol dicaprylocaprate), and Plurol Oleique CC 497 (polyglyceryl oleate), were kindly supplied by Gattefossé Canada, Inc. (Toronto, ON, Canada). Cetyl alcohol and xanthan gum were obtained from Medisca Pharmaceutique Inc. (Montreal, QC, Canada). Glycerin and mineral oil were purchased from Mallinckrodt Specialty Chemical Company (Paris, KY, USA). Acetonitrile, glacial acetic acid, potassium phosphate monobasic, sodium hydroxide, and castor oil were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Polyoxyethylene 20-oleyl ether (Brij<sup>®</sup> 98) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Arlacel P135 was supplied by UNIQEMA (New Castle, DE, USA). Cabomer 934 was obtained from BF Goodrich Company, Chemical Group (Cleveland, OH, USA). Low-density polyethylene (LDPE) membrane was supplied by Key Container (Winnipeg, MB, Canada).

### 2.2. Emulsion formulations

Three emulsion-based lotions were prepared according to formulas outlined in Table 1. In preparing Formulation A (FA), Cabomer 934 was allowed for natural swelling in water at room temperature for 24 h. Phase I (oil phase) including 5% OBZ and Phase II (aqueous phase) were separately heated to 75 °C at 500 rpm. Phase I was then poured into Phase II at 1500 rpm; the mixture was agitated for 15 min. Sodium hydroxide (10%, 0.5 mL) was

**Table 1** Emulsion-based formulations design.

FA Oil in Water	FB Oil in Water with Viscosity	FC Water in Oil
<b>I</b> Emulfree CBG/4% Labrasol/8% Geleol/2% Cetyl alcohol/4%	<b>I</b> Emulfree CBG/4% Labrasol/8% Geleol/2% Cetyl alcohol/4%	<b>I</b> Arlacel P135/3% Geleol/3% Isostearate isostearyl/5% Cetyl alcohol/10% Mineral oil/9%
<b>II</b> Demineralized water/QS Glycerin/5% Carbomer 934/0.25%	<b>II</b> Demineralized water/QS Glycerin/5% Carbomer 934/0.25%	<b>I</b> Labrasol/7% Labrafac PG/8% Caster oil/5%
<b>III</b> Sodium hydroxide sol (10%)/0.5%	Xanthan gum/0.4% <b>III</b> Sodium hydroxide sol (10%)/0.5%	Plurol oleique CC 497/5% <b>II</b> Demineralized water/35% Glycerin/5%

added to the mixture for pH adjustment. DEET (7.5%) was added during congealing of the emulsion; the emulsion was further agitated until cooled down to room temperature. Preparation of Formulation B (FB) was similar to that of FA, except that xanthan gum was added to the emulsion as a thickening agent. In preparing Formulation C (FC), Phase I (oil phase) containing 5% OBZ and Phase II (aqueous phase) were initially heated to 75 °C under agitation. Phase II was then added to Phase I; the mixture was agitated for 15 min. DEET (7.5%) was added during congealing of the emulsion; agitation was continued until the preparation was cooled down to room temperature.

The prepared lotions were transferred to sealed plastic containers and stored at room temperature for one month. Droplet size distribution was observed and recorded on the last day of storage.

### 2.3. Droplet size distribution

The surface morphology of the emulsion droplets was observed and recorded using an optical microscope fitted with a digital camera (James Swift MP3502, Prior Scientific Instruments Ltd.). A drop of the diluted emulsion was placed on a microscope slide, and a piece of glass placed over the drop. Observations were made afterwards.

The droplet size of the emulsions was also measured using a Malvern Mastersizer 2000s (Malvern Instruments Ltd., Malvern, Worcestershire, UK). In case of laser diffraction measurements, one drop of o/w emulsion was suspended in the dispersion accessory with distilled water, which was continuously homogenized at 12,000 rpm and ultrasonicated for 90 s prior to measurement. For w/o emulsion, acetone was added to the dispersion accessory as a dispersant. Correction measurements were also taken to compensate for background electrical noise and laser scattering interferences from optics or samples. Three replicates were run for each emulsion sample. A typical analysis interval was 3 min per sample after optical alignment and background measurement. Raw data was analyzed using Malvern software; the mean droplet diameter was mathematically expressed as the following equation,

$$D[4,3] = \frac{\sum D_i^4 N_i}{\sum D_i^3 N_i} \quad (1)$$

where  $D[4,3]$  is the volume weighted mean,  $D_i$  is the diameter of particles and  $N_i$  is the number of particles.

### 2.4. Human skin and artificial LDPE

Human skin specimens were obtained from St. Boniface General Hospital of Winnipeg and stored at  $-20$  °C prior to use. The study protocol was approved by the University of Manitoba Human Research Ethics Board. To prepare skin samples for diffusion experiments, the skin was taken out from the freezer and thawed at room temperature for approximately 8 h. The specimens were then dermatomed (Padgett Instruments, Kansas City, MO, USA) to a thickness of 380  $\mu$ m and soaked in saline solution to prevent membrane from dehydration. The undamaged skin section with an even thickness was selected for diffusion experiments, and was cut into small pieces of 2 cm  $\times$  2 cm before being mounted to Franz-style diffusion cells.

Artificial low-density polyethylene (LDPE, Key Container, Winnipeg, MB, Canada) membrane was also used for diffusion experiments; this membrane model possessed lipophilic properties. LDPE membrane was cut into small pieces of 2 cm  $\times$  2 cm, and soaked in saline solution prior to being mounted to vertical Franz-style diffusion cells.

### 2.5. Diffusion study

*In vitro* diffusion experiments were conducted in a transdermal diffusion cell console (Logan Instruments Corporation, Somerset, NJ, USA), which was composed of six vertical Franz-style diffusion cells, a circulated water bath, a magnetic stir console and an automatic sampling collector. Prior to the experiment, a very thin layer of vacuum grease was spread on the connection surface of the receptor and donor cells to avoid leakage of the samples. Phosphate buffer (pH 7.4 containing 4% Brij 98) was filled into the receptor cell. LDPE membrane or human skin with stratum corneum facing up was mounted to the connection surface of the receptor cell. The donor cell was put on the membrane; and both receptor and donor cells were tightly fixed by a clamp. Emulsion sample (1.0 g) was accurately weighed and carefully applied into the donor cell, so that a complete contact between the emulsion and the membrane surface was maintained. The donor cell was covered with a piece of microscope glass to prevent evaporation of the sample. Water bath and magnetic stir console were turned on and respectively maintained at 37 °C and 300 rpm. The amount of emulsion applied into the donor cell that was in direct contact with the membrane surface and adjacent effective diffusion area was precisely measured to be 0.1 g, which was used to calculate the overall permeation percentage of DEET and OBZ.

An aliquot of receptor fluid was collected hourly for 6 h, followed by replenishing an equal volume of fresh, preheated receptor fluid at each sampling point. Four test replicates were performed in each experiment. Concentration of DEET and OBZ in the receptor fluid was directly analyzed without further treatment by an HPLC method that had been previously developed and validated. After the diffusion experiment, each individual LDPE membrane or human skin was respectively collected into a small glass vial, and soaked in 20 mL acetonitrile for 24 h at room temperature. The acetonitrile solution was centrifuged for 15 min afterwards. The supernatant was transferred and also analyzed using the HPLC assay.

### 2.6. HPLC assay

All study samples were analyzed using an HPLC system composed of a 996 Photodiode Array Detector and a Waters Alliance 2690 Solvent Delivery Module with Millennium software (Milford, MA, USA). The column was Nova-Pak C18, 150 mm  $\times$  3.9 mm, 4  $\mu$ m. The mobile phase was a mixture of acetonitrile, methanol and water (pH 2.8 with acidic acid) in the ratio of 65:20:15 (v:v:v), delivered at a flow rate of 1 mL/min. The detection wavelength of DEET was 254 nm and that of OBZ was 287 nm. Under these conditions, the retention time of DEET and OBZ was 1.49 min and 1.98 min respectively, with a detection limitation of 800 ng/mL DEET and 200 ng/mL OBZ. The range of calibration linearity ( $r^2 \geq 0.99$ ) for DEET and OBZ was 2–80  $\mu$ g/mL and 1–20  $\mu$ g/mL, respectively.

### 2.7. Data analysis

Fick's Second Law of Diffusion is commonly utilized to quantitate drug diffusion characteristics across skin membrane, which is expressed as the following<sup>10</sup>,

$$\frac{\partial C_v}{\partial t} = D_m \frac{\partial^2 C_m}{\partial x^2} \quad (2)$$

where  $C_m$  is drug concentration in membranes (g/mL) and  $D_m$  is diffusion coefficient ( $\text{cm}^2/\text{h}$ ).

Above equation presumes that (a) the concentration of a solute in the donor cell remains constant; (b) "sink condition" exists in the receptor cell for the duration of the experiment; and (c) the initial concentrations in membrane model are  $C_m(x,0)=0$ ,  $C_m(0,t)=K_m C_v$ , and  $C_m(h_m, t)=0$ . Under most circumstances, Fick's Second Law is solved in terms of the amount of solute  $Q(t)$  exiting from the membrane at time  $t$ , and is expressed as the following<sup>10</sup>,

$$\frac{Q(t)}{A} = K_m h_m C_v \left[ \frac{D_m t}{h_m^2} - \frac{1}{6} - \frac{2}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-\frac{D_m \pi^2 n^2 t}{h_m^2}\right) \right] \quad (3)$$

where  $Q(t)$  is accumulative drug amount permeating through the membrane (g),  $K_m$  is partition coefficient between membrane and vehicle,  $C_v$  is saturated drug concentration in vehicle (g/mL),  $h_m$  is thickness of the membrane (cm),  $A$  is diffusion area ( $\text{cm}^2$ ), and  $D_m$  is diffusion coefficient ( $\text{cm}^2/\text{h}$ ).

When  $t \rightarrow \infty$ , Eq. (3) is simplified to the following<sup>10</sup>,

$$\begin{aligned} \frac{Q(t)}{A} &= K_m h_m C_v \left( \frac{D_m t}{h_m^2} - \frac{1}{6} \right) \\ &= \frac{K_m C_v D_m}{h_m} \left( t - \frac{h_m^2}{6 D_m} \right) \\ &= K_p C_v (t - \text{Lag}) \end{aligned} \quad (4)$$

where permeability coefficient  $K_p$  of a solute is expressed in

$$K_p = \frac{K_m D_m}{h_m} \quad (5)$$

Lag time is given by

$$\text{Lag} = \frac{h_m^2}{6 D_m} \quad (6)$$

Permeation percentage can be calculated as the following,

$$\text{Permeation percentage} = \frac{Q(t)}{\text{Amount of active ingredients in emulsion samples}} \quad (7)$$

Once a steady-state status is reached in percutaneous penetration, the permeation parameters can also be calculated by Eq. (4) using linear regression of the experimental data. In this linear equation,

$$\text{Slope} = \frac{K_m C_v D_m}{h_m} \quad (8)$$

$$\text{Intercept} = \frac{K_m C_m h_m}{6} \quad (9)$$

Subsequently, permeation parameters can be calculated as the following,

$$K_p = \frac{6 \cdot \text{Intercept} \cdot \text{Slope}}{C_v^2 K_m} \quad (10)$$

Statistical analysis was performed using two-way ANOVA and Tukey's Test (PC-SAS 8.02, SAS Institute Inc., Cary, North Carolina, USA). The statistical analyses were conducted on the data of the overall permeation percentages and permeation coefficient of DEET and OBZ through LDPE and human skin. Differences were considered statistically significant at  $P \leq 0.05$ .

### 3. Result and discussion

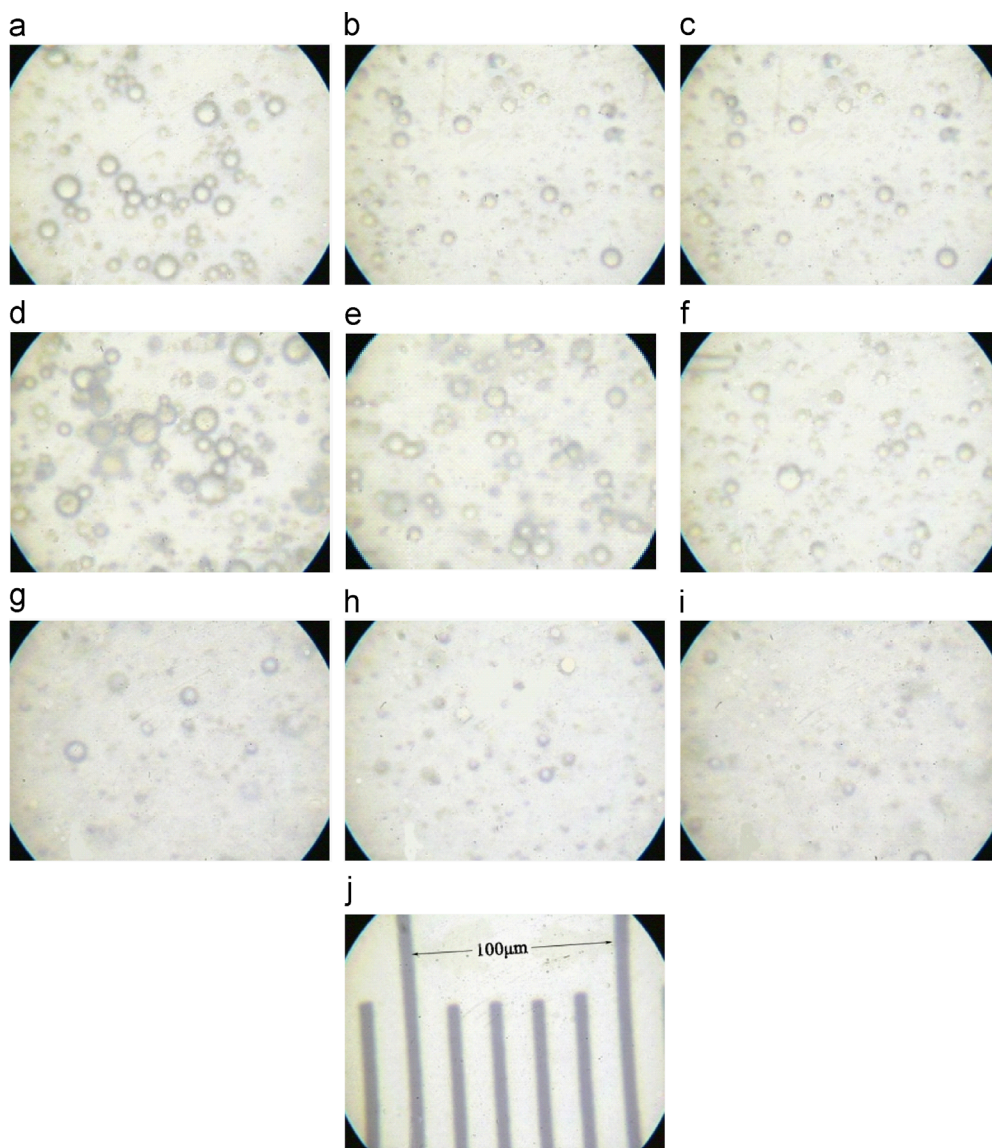
#### 3.1. Droplet surface morphology

The surface morphology of emulsion droplets was observed using an optical microscopy; pictures were taken using a digital camera. As shown in photomicrographs (Fig. 1), all three emulsion formulations were simple emulsions. Oil globules dispersed throughout continuous aqueous phase in FA and FB, while aqueous globules dispersed throughout continuous oil phase in FC. The oil and aqueous globules were found to be spherical with smooth surface. In FA containing DEET or OBZ, the oil globules appeared to be uniformly distributed within the continuous phase. Aggregation of oil globules was observed in FA containing combined DEET/OBZ, which may have resulted in larger droplet size. FB containing DEET or OBZ also showed uniform distribution of oil globules within continuous phase, while FB containing combined DEET/OBZ exhibited aggregation of the oil droplets. In FC, all preparations produced uniform distribution of aqueous globules in continuous oil phase without evidence of globule aggregation.

#### 3.2. Droplet size distribution

Droplet size of the prepared emulsions were measured using a laser diffraction machine. Table 2 shows the volume mean diameter of droplets. The droplet size of FB containing DEET or OBZ was significantly different from FA counterparts. The volume mean diameter of droplets in FB containing DEET was significantly smaller than that in FA by 16%, while the volume mean diameter of FB containing OBZ was significantly larger than FA counterpart by 43%. Adding thickening agent xanthan gum was able to increase viscosity of aqueous phase when its concentration reached beyond 0.2%, which led to decrease in droplet diameter<sup>11</sup>. In this study, adding xanthan gum only reduced droplet size in o/w emulsion containing DEET, but not in o/w emulsion containing OBZ or combined DEET/OBZ. Oil globules containing OBZ demonstrated different physical properties when compared to DEET counterpart, which might have induced different coalescent abilities of droplets in o/w emulsions containing thickening agent. Oil droplets containing OBZ tended to coalesce and form larger droplets in o/w emulsion when thickening agent was present, but oil droplets containing DEET aggregated slowly and maintained smaller droplets size. Furthermore, droplet size of FA and FB containing combined DEET/OBZ was significantly larger than those containing DEET or OBZ. FA containing combined DEET/OBZ had significantly larger droplet size by 33% or by 51% than FA containing DEET or OBZ, respectively. FB containing combined DEET/OBZ produced significantly higher droplet size by 12% or by 67% when compared to FB containing DEET or OBZ, respectively. The higher droplet size in the combined preparations of o/w emulsion may be attributed to possible interaction between DEET and OBZ within oil droplets, which would subsequently compromise stability of oil droplets containing combined DEET/OBZ. Previous observation in photomicrographs (Fig. 1) showed that the oil droplets containing combined DEET/OBZ tended to aggregate in o/w emulsion. This result was confirmed by observation of droplets size in laser diffraction machine.

In addition, FC containing DEET, OBZ, or both possessed significantly lower volume mean diameter of droplets than FA counterparts, by 40%, 50% and 45%, respectively. The droplet size in FC containing combined DEET/OBZ did not display significant difference from that containing DEET or OBZ. The different droplet



**Figure 1** The appearance of droplets in FA, FB, FC. The photomicrographs of three emulsion-based formulations with DEET and/or OBZ (a: FA with DEET and OBZ; b: FA with OBZ; c: FA with DEET; d: FB with DEET and OBZ; e: FB with OBZ; f: FB with DEET; g: FC with DEET and OBZ; h: FC with OBZ; i: FC with DEET; j: Scale) were obtained using an optical microscopy and a digital camera. All the preparations are simple emulsions (oil-in-water or water-in-oil).

sizes between w/o emulsion and o/w emulsion may be attributed to different emulsifiers used in the two emulsions. In o/w emulsion, emulsifier formed multilayer on the interface of water and oil phases to stabilize oil droplets by steric repulsion, rather than their negligible ability to lower interfacial tension<sup>12</sup>. In w/o emulsion, the nonionic synthetic emulsifier formed a coherent interfacial film surrounding the dispersed droplets to lower the interfacial tension of droplets and provided the system a high degree of resistance to coalescence. Aqueous droplets in w/o emulsion may coalesce slowly and retain a smaller droplet diameter comparing to oil droplets in the o/w emulsion.

### 3.3. Permeation of DEET and OBZ through human skin

Figs. 2 and 3 show overall permeation percentage of DEET and OBZ within membrane and receptor fluid from FA, FB, and FC

respectively. Comparing FB to FA in single preparation, overall permeation percentage of DEET and OBZ (including both membrane and receptor fluid) significantly increased by 21% and 150%; permeation percentage of OBZ within membrane was significantly higher by 400%. Combined preparation of FB and OBZ significantly increased overall permeation percentage of DEET and OBZ by 36% and 23% comparing to its single preparations; no synergistic permeation of DEET and OBZ was observed in combined preparation of FA. Permeation percentage of DEET and OBZ within membrane was significantly increased by 1360% and 67% in the combined preparation of FB in comparison to its single preparation. The higher skin permeation of DEET and OBZ and synergistic percutaneous permeation of the two compounds in FB may be attributed to the addition of thickening agent in the external phase of the o/w emulsion. Xanthan gum was added to the dispersing phase to increase homogeneity and stability of the emulsion. The addition of xanthan gum may increase stability of

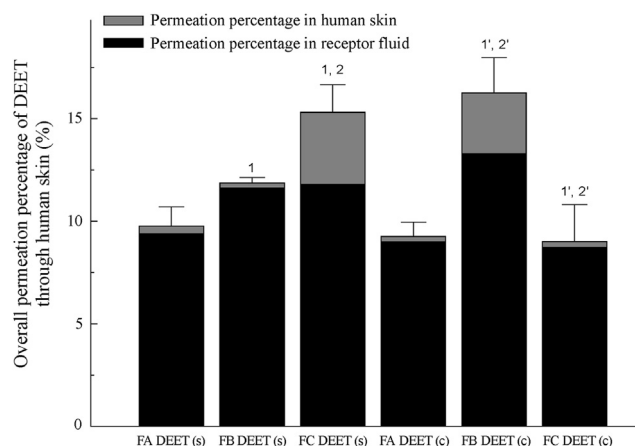
**Table 2** The volume mean diameters of droplet ( $D$  [4,3],  $\mu\text{m}$ ).

Active ingredient	Formulation		
	FA	FB	FC
DEET (S)	58.43 $\pm$ 1.62	49.16 $\pm$ 0.71 <sup>a</sup>	35.15 $\pm$ 0.39 <sup>a</sup>
OBZ (S)	51.26 $\pm$ 0.41	73.18 $\pm$ 1.12 <sup>a</sup>	27.06 $\pm$ 0.30 <sup>a</sup>
OBZ (C)	77.51 $\pm$ 1.23 <sup>a,b</sup>	82.18 $\pm$ 0.79 <sup>a,b</sup>	32.62 $\pm$ 1.12 <sup>a</sup>

Data are expressed as Mean  $\pm$  SD,  $n=3$ ,  $P \leq 0.05$ .

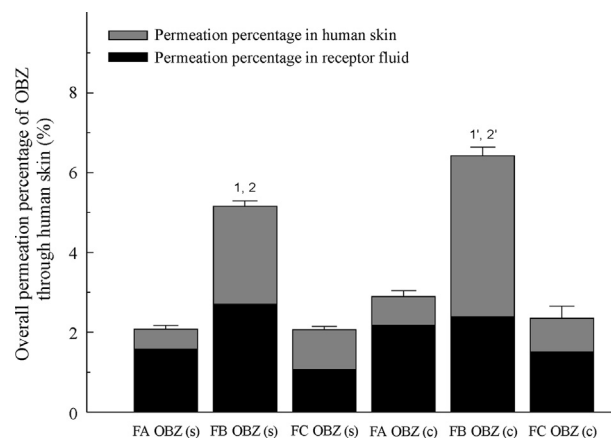
<sup>a</sup>Significant difference between FA and FB or between FA and FC.

<sup>b</sup>Significant difference between single preparations and combined preparations.



**Figure 2** Overall DEET permeation percentage from three formulations through human skin ( $n=4$ , Mean  $\pm$  SEM,  $P \leq 0.05$ ). 1: Significant difference between FA and FB/FC (membrane+receptor fluid); 1': Significant different between single preparations and combined preparations (membrane+receptor fluid); 2: Significant difference between FA and FB/FC (membrane only); 2': Significant difference between single preparations and combined preparations (membrane only).

the oil droplets and reduce diameter of the oil droplets<sup>11</sup>. In FB, the diameter of oil droplets containing DEET was significantly reduced (Table 2). The smaller droplet size increased the surface area of oil droplets and enhanced the contact area of oil droplets with skin surface, which may subsequently induce higher skin permeation of the active ingredients, such as DEET, from an emulsion<sup>7,13</sup>. However, the diameter of oil droplets containing OBZ was not decreased, as oil droplets containing OBZ tended to coalesce and formed larger oil droplets. Although FB containing OBZ had larger oil droplet size, permeation of OBZ from FB still significantly increased comparing to FA. This implied that xanthan gum may increase OBZ affinity to the membrane and hence facilitate OBZ partition into the skin from oil droplets, thereby increasing membrane retention and permeation of OBZ as shown in Fig. 3. Furthermore, concurrent presence of DEET and OBZ in FB together with xanthan gum induced synergistic skin retention and permeation of the two compounds, which may also be attributed to the addition of xanthan gum, since it increased contact time for drug diffusion.



**Figure 3** Overall OBZ permeation percentage from three formulations through human skin ( $n=4$ , Mean  $\pm$  SEM,  $P \leq 0.05$ ). 1: Significant difference between FA and FB/FC (membrane+receptor fluid); 1': Significant difference between single preparations and combined preparations (membrane+receptor fluid); 2: Significant difference between FA and FB/FC (membrane only); 2': Significant difference between single preparations and combined preparations (membrane only).

FC significantly increased the overall permeation percentage of DEET in single preparation by 56% compared to FA, while permeation percentage of DEET within membrane was significantly higher by 9.6-fold in single preparation of FC than FA. There was no significant difference in overall permeation percentage of OBZ between FC and FA. The combined preparation of FC resulted in a decrease of 41% in overall permeation of DEET comparing to its single preparation, but there was no significant difference in OBZ permeation between combined and single preparations. Skin permeation of active ingredients from a w/o emulsion may be influenced by solubility capacity of a compound in both organic and aqueous phases. DEET and OBZ are both lipophilic compounds with log  $K_{o/w}$  (octanol/water partition coefficient) of 2.01 and 3.79, respectively. They were dissolved in oil phase of the emulsion, which was the external phase in FC. When a substance was dissolved in the external phase of an emulsion, it may have a closer contact with interfacial area between membrane and vehicle, and hence produce higher skin permeation than a chemical dissolved in the internal phase<sup>14</sup>. DEET, a lipophilic compound, showed higher skin permeation from the w/o emulsion FC2 than from the o/w emulsion FA. In addition, certain oil components of the external phase, such as castor oil, may also permeate into stratum corneum<sup>15</sup>, and increase the solubility of DEET in the skin, resulting in higher skin retention of DEET in FC. However, OBZ is more lipophilic than DEET. In accordance with the law of "like dissolves like", OBZ may possess stronger affinity to highly lipophilic oil components in the external phase than with stratum corneum of human skin, so that OBZ may demonstrate lower driven force in FC to permeate through human skin. Therefore, OBZ did not produce a significant increase of skin permeation from FC when compared to FA, even though OBZ was dissolved in the external phase of FC. Similar results had also been reported that skin permeation of lipophilic parabens was enhanced from o/w emulsions compared to w/o emulsions<sup>16</sup>. Furthermore, presence of OBZ in FC may increase the affinity of DEET for the oil components in FC, thereby decreasing skin permeation of DEET from the preparation.

Permeation coefficient is an important parameter in skin diffusion and penetration; it describes the velocity at which a permeant travels across a membrane. Permeation coefficient is often utilized to compare permeation profiles of a substance, which is examined under different experimental conditions. Permeability of an active ingredient can be influenced by carrier vehicle and interaction between the active ingredient and/or vehicle/skin<sup>6</sup>. Emulsion type and excipients present in the emulsion frequently play important roles in modifying permeability of the active ingredients through membrane models. Table 3 lists permeation coefficients of DEET and OBZ in FA, FB, and FC through human skin. Among the three emulsions, permeation coefficients through human skin ranged between 16.06 and 30.07 ( $\times 10^{-4}$ , cm/h) for DEET and 2.03 and 4.67 ( $\times 10^{-4}$ , cm/h) for OBZ. FB significantly increased DEET permeation coefficient through human skin by 22% when compared to FA. This indicated that increase in DEET permeability from o/w emulsion containing single DEET might be related to decrease of droplet size in emulsion formula (FB) with thickening agent xanthan gum. Concurrent presence of DEET and OBZ in FB significantly increased DEET permeation coefficient by 7%, which indicated that OBZ might also increase DEET permeability in o/w emulsion containing xanthan gum. This may be partially attributed to interactions between xanthan gum and the two compounds within both vehicle and membrane. FC did not result in increase of DEET permeation coefficient through human skin when compared to FA, while presence of OBZ in FC significantly decreased DEET permeation coefficient by 27%. Since OBZ may exhibit higher affinity for oil components in a w/o emulsion, its permeability may be reduced as a result. OBZ may also enhance affinity of DEET for oil phase in a w/o emulsion, and hence decrease DEET permeability through human skin. Previous studies had indicated that permeation of DEET and OBZ was dependent upon formulation types and excipients<sup>17–19</sup>, which were in agreement with findings from this study. In addition, there was no significant difference observed in permeation coefficient of OBZ through human skin.

### 3.4. Permeation of DEET and OBZ through LDPE

Figs. 4 and 5 show the overall permeation percentage of DEET and OBZ within membrane and receptor fluid from FA, FB, and FC through LDPE. Overall permeation percentage of OBZ through

**Table 3** Permeation coefficient of DEET and OBZ through human skin ( $\times 10^{-4}$ , cm/h).

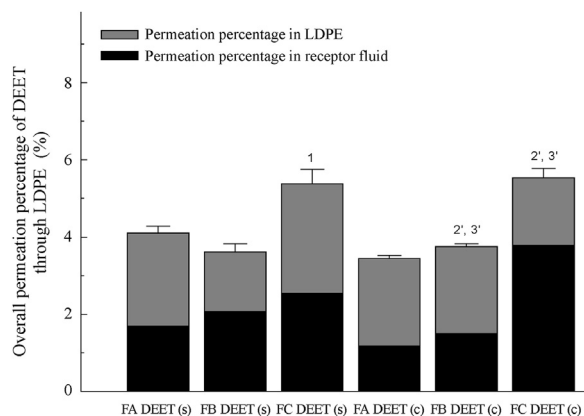
Active ingredient	Formulation		
	FA	FB	FC
DEET (S)	23.31 $\pm$ 1.95 <sup>a,b</sup>	27.95 $\pm$ 0.76 <sup>a,b,c</sup>	22.09 $\pm$ 2.57 <sup>a,c</sup>
DEET (C)	20.95 $\pm$ 2.00 <sup>a</sup>	30.07 $\pm$ 3.95 <sup>a,c</sup>	16.06 $\pm$ 2.47 <sup>a,c</sup>
OBZ (S)	3.09 $\pm$ 0.23 <sup>a</sup>	3.77 $\pm$ 0.50 <sup>a</sup>	2.03 $\pm$ 0.20 <sup>a</sup>
OBZ (C)	4.09 $\pm$ 0.31 <sup>a</sup>	4.67 $\pm$ 0.40 <sup>a</sup>	2.16 $\pm$ 0.30 <sup>a</sup>

Data are expressed as Mean  $\pm$  SEM,  $n=4$ ,  $P \leq 0.05$ .

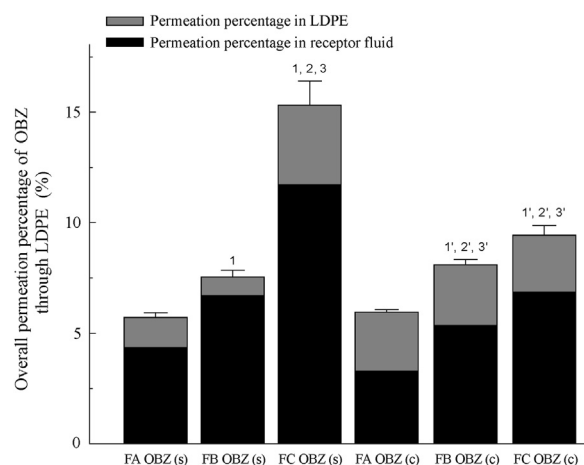
<sup>a</sup>Significant difference between LDPE and human skin.

<sup>b</sup>Significant difference in combined DEET between FA and FB/FC.

<sup>c</sup>Significant difference between single preparations and combined preparations.



**Figure 4** Overall DEET permeation percentage from three formulations through LDPE ( $n=4$ , Mean  $\pm$  SEM,  $P \leq 0.05$ ). 1: Significant difference between FA and FB/FC (membrane+receptor fluid); 1': Significant difference between single preparations and combined preparations (membrane+receptor fluid); 2: Significant difference between FA and FB/FC (membrane only); 2': Significant difference between single preparations and combined preparations (membrane only); 3: Significant difference between FA and FB/FC (receptor fluid only); 3': Significant different between single preparations and combined preparations (receptor fluid only).



**Figure 5** Overall OBZ permeation percentage from three formulations through LDPE ( $n=4$ , Mean  $\pm$  SEM,  $P \leq 0.05$ ). 1: Significant difference between FA and FB/FC (membrane+receptor fluid); 1': Significant difference between single preparations and combined preparations (membrane+receptor fluid); 2: Significant difference between FA and FB/FC (membrane only); 2': Significant difference between single preparations and combined preparations (membrane only); 3: Significant difference between FA and FB/FC (receptor fluid only); 3': Significant different between single preparations and combined preparations (receptor fluid only).

LDPE was significantly increased by 26%–628% comparing to that of OBZ through human skin. At the same time, overall permeation percentage of DEET through human skin was significantly increased by 64%–338% comparing to that of DEET through LDPE. These variable permeation profiles between DEET

and OBZ may result from membrane differences and interactions between chemicals and membranes. LDPE is an artificial membrane made of low-density polyethylene resin, and possesses lipophilic properties in nature. Human skin is much more structurally complex than LDPE. Stratum corneum, a principal rate-limiting barrier against percutaneous permeation, is comprised of lipophilic components (intercellular lipid matrix) and hydrophilic components (corneocytes)<sup>20</sup>. DEET may create a proper balanced affinity for both lipid components and hydrophilic keratin parts in stratum corneum due to its appropriate  $\log K_{o/w}$  2.01 and low molecular weight, hence permeating more through human skin than through LDPE. With a higher  $\log K_{o/w}$  of 3.79, OBZ was more lipophilic and possessed a higher affinity for LDPE than for human skin; as such its permeation through LDPE was higher than DEET.

It was also noted in Figs. 4 and 5 that FB did not increase overall permeation percentage of DEET in single preparation when compared to FA. Presence of OBZ in FB significantly increased DEET retention within membrane by 120% and decreased its permeation into receptor fluid by 29%, but it did not significantly increase overall permeation of DEET. Adding xanthan gum to the emulsion may form smaller droplet size in the preparation containing single DEET, but it apparently did not alter DEET affinity for more lipophilic membrane LDPE. The lower affinity of DEET for LDPE membrane became a key factor in modifying its membrane permeation from different formulations. In addition, OBZ may help increase DEET solubility within membrane; xanthan gum, as a lipophilic long-chain macromolecule, may also combine with lipophilic polymer membrane LDPE and increase stability of DEET within the membrane. Therefore, DEET was retained more in the membrane and diffused less into the receptor fluid. For OBZ, FB significantly increased the overall permeation percentage in single preparation by 32% when compared to FA. The combined FB preparation significantly increased the overall permeation percentage of OBZ by 8% in comparison to its single preparation; it also significantly increased OBZ retention within membrane by 240% and significantly decreased OBZ permeation into receptor fluid by 19% when compared to its single preparation. Presence of DEET together with xanthan gum in FB may have increased OBZ affinity and stability within more lipophilic membrane LDPE, leading to higher retention of OBZ within the membrane.

For FC, overall permeation percentage of DEET in single preparation significantly increased by 31% comparing to FA. Presence of OBZ in FC significantly increased DEET permeation percentage in the receptor fluid by 46%, and decreased its retention within the membrane by 36%. However, no significant difference was observed in the overall permeation percentage of DEET. With a w/o emulsion, DEET was dissolved in the external oil phase. Chemicals that are dissolved in external phase would have higher skin permeation<sup>6</sup>. Therefore, DEET in external phase of a w/o emulsion demonstrated higher membrane permeation than in internal phase of an o/w emulsion. In addition, the more lipophilic OBZ possessed higher affinity for lipophilic membrane LDPE than DEET. Due to concurrent presence of DEET and OBZ, OBZ may facilitate DEET affinity for LDPE and increase DEET diffusion within membrane, which consequently supplied higher driving force for DEET to diffuse into the receptor fluid and to enhance its transmembrane permeation. For OBZ, its overall permeation percentage in single FC preparation was significantly higher by 169% than FA. This was higher by 180% within the membrane and by 52% in the receptor fluid. Presence of DEET significantly decreased overall OBZ permeation by 40% within the membrane

**Table 4** Permeation coefficient of DEET and OBZ through LDPE ( $\times 10^{-4}$ , cm/h).

Active ingredient	Formulation		
	FA	FB	FC
DEET(S)	4.45 ± 0.48 <sup>a</sup>	5.18 ± 0.56 <sup>a</sup>	5.77 ± 0.87 <sup>a</sup>
DEET(C)	3.13 ± 0.21 <sup>a</sup>	3.93 ± 0.19 <sup>a</sup>	8.80 ± 0.59 <sup>a</sup>
OBZ (S)	10.61 ± 0.60 <sup>a,b</sup>	14.65 ± 0.64 <sup>a,b,c</sup>	26.75 ± 2.64 <sup>a,b,c</sup>
OBZ (C)	7.97 ± 0.30 <sup>a</sup>	11.80 ± 0.54 <sup>a,c</sup>	13.20 ± 0.78 <sup>a,c</sup>

Data are expressed as Mean ± SEM,  $n=4$ ,  $P \leq 0.05$ .

<sup>a</sup>Significant difference between LDPE and human skin.

<sup>b</sup>Significant difference in single OBZ between FA and FB/FC.

<sup>c</sup>Significant difference between single preparations and combined preparations.

and by 36% in the receptor fluid. In w/o emulsion, a lipophilic OBZ was also dissolved in the external phase; it possessed a higher affinity for LDPE. These may contribute to higher driving force for OBZ to release into the membrane and to diffuse into the receptor fluid, thereby increasing its membrane retention and transmembrane permeation. Compared to OBZ, DEET had a lower affinity for lipophilic LDPE. Possible interaction between DEET and OBZ may affect OBZ affinity to LDPE. Thus OBZ membrane retention and transmembrane permeation were reduced.

Permeation coefficients of DEET and OBZ in FA, FB, and FC through LDPE are listed in Table 4, they ranged between 3.13 and 8.80 ( $\times 10^{-4}$ , cm/h) for DEET and 7.97 and 26.75 ( $\times 10^{-4}$ , cm/h) for OBZ, respectively. OBZ permeability through LDPE was increased in comparison to human skin, but that of DEET was decreased. This may be attributed to higher affinity of OBZ to lipophilic LDPE and higher affinity of DEET to human skin. When comparing permeation coefficient of DEET and OBZ among the three prepared emulsions, FB significantly increased OBZ permeability in single preparation by 39% in comparison to FA. OBZ permeability in combined preparation of FB was significantly decreased by 20% in comparison to single preparation of FB. Adding xanthan gum as thickening agent apparently led to higher OBZ permeability, while presence of DEET reduced OBZ permeability. These were consistent with results observed in OBZ permeation percentage from FB preparations. Permeation coefficient of OBZ through LDPE in single FC preparation showed a significant increase of 152% in comparison to that of FA. Permeation coefficient of OBZ through LDPE in combined FC preparation was significantly lowered by 51% in comparison to its single preparation. This implied that OBZ dissolved in the external oil phase exhibited higher permeability in FC. Presence of DEET decreased OBZ permeability due to reduced affinity of OBZ for LDPE membrane. In addition, there was no significant difference observed in DEET permeation through LDPE.

#### 4. Conclusions

Formulation design and preparation are critical in topical drug delivery; emulsion-based preparations are commonly selected for their excellent solubilizing properties to accommodate lipophilic and hydrophilic compounds, and their satisfactory stability and acceptability. Designing and adjusting emulsion parameters could subsequently modify skin permeation of the active ingredients



from these dosage forms. Percutaneous permeation of insect repellent DEET and sunscreen OBZ in three emulsion-based lotions through artificial membrane LDPE and human skin was found to be dependent on the emulsion type, the addition of thickening agent and the internal droplet size.

O/w emulsions demonstrated desirable potential of lowering percutaneous permeation of DEET through human skin in comparison to w/o emulsions from *in vitro* diffusion experimentation. Furthermore, adding xanthan gum to o/w emulsion reduced the size of oil droplets containing DEET and increased percutaneous permeation of DEET through human skin; xanthan gum in the o/w emulsion containing DEET and OBZ also produced synergistic permeation of the two compounds through human skin.

Percutaneous permeation of OBZ through LDPE membrane was significantly increased in comparison to human skin; on the other hand, percutaneous permeation of DEET through human skin was significantly higher than that through LDPE. Adding xanthan gum to o/w emulsion led to synergistic retention of the two compounds in artificial membrane LDPE. The w/o emulsion increased permeation of DEET and OBZ through LDPE; concurrent presence of the two compounds in a w/o emulsion also induced synergistic transmembrane permeation through LDPE.

While artificial membrane LDPE produced different permeation profiles in comparison to human skin model, this artificial membrane model could still be considered a reliable option for evaluating percutaneous permeation of topical formulations in quality control when human skin specimens are unavailable.

### Acknowledgment

The authors wish to acknowledge research support from Canada Foundation for Innovation (CFI) and Manitoba Institute of Child Health (MICH), and graduate studentship from MHRC (TW). Generous supply of testing materials from Gattefossé Canada, Key Container of Winnipeg, and Hydranautics of Oceanside is also acknowledged.

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