Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 310 (2006) 110–117
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

In vitro permeation of repellent DEET and sunscreen oxybenzone across three artificial membranes

Tao Wang, Sreeneeranj Kasichayanula, Xiaochen Gu *

Faculty of Pharmacy, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

Received 28 April 2005; received in revised form 28 November 2005; accepted 28 November 2005
Available online 18 January 2006

Abstract

DEET and oxybenzone are two essential active ingredients in repellent and sunscreen products. We performed a series of in vitro diffusion studies to evaluate the transmembrane permeation of DEET and oxybenzone across three artificial membranes, low-density polyethylene (LDPE), low fouling composite (LFC) and mixed cellulose esters (MCE), from concurrent use of commercial repellent and sunscreen preparations. Permeation of DEET and oxybenzone across the test membranes was synergistically increased when both the repellent and the sunscreen formulations were applied simultaneously. Different application sequences and formulation types also resulted in variable permeation profiles of DEET and oxybenzone. Compared to biological piglet epidermis under the identical experimental conditions, transmembrane permeation of DEET was suppressed in LDPE and LFC membranes, but enhanced in MCE membrane; transmembrane permeation of oxybenzone was reduced in LFC membrane, but increased in LDPE and MCE membranes. Permeability coefficients of DEET and oxybenzone in all three artificial membranes were significantly different from those in piglet skin. It was concluded that the permeation profiles of the compounds were dependent upon physicochemical characteristics of the membranes and the formulations.

Keywords: Diffusion; Membrane transport; Permeability; Concurrent use; Insect repellents; Sunscreens

1. Introduction

Insect repellents and sunscreens have been extensively used as over-the-counter consumer care products by the general public to prevent vector-borne diseases and sunburns. Before 1999, concurrent application of repellent and sunscreen preparations was rare, since biting insects such as mosquitoes are likely to be more active during dawn and dusk when the ultraviolet (UV) radiation from sunlight is not strong enough for the use of sunscreens. However, with the arrival of West Nile virus in North America and its prompt dissemination by mosquitoes, concurrent use of repellents and sunscreens for outdoor activities has been widely practiced by many for fear of contacting the lethal virus, especially in mosquito-infested regions and areas.

DEET (*N*,*N*-diethyl-*m*-toluamide) has been used as one of the most effective insect repellents for decades (Fradin, 1998; Staub et al., 2002; Koren et al., 2003). Oxybenzone is one of the primary UVA/UVB sun-blocking agents present in many commercially available sunscreen preparations (Chatelain et al., 2003; Felton et al., 2004). Transdermal permeation of DEET and oxybenzone from topical application has been studied and reported separately (Hayden et al., 1997; Qiu et al., 1997; Abdel-Rahman et al., 2004; Pont et al., 2004). Influence of co-applying repellents and sunscreens on the efficacy of repellency and UV protection was also investigated (Montemarano et al., 1997; Murphy et al., 2000). We have recently reported a synergistic percutaneous penetration of DEET and oxybenzone when both components were used simultaneously (Gu et al., 2004, 2005; Kasichayanula et al., 2005).

The use of Franz-style diffusion cells in in vitro transdermal drug evaluations has been well established and documented (Higuchi, 1967; Bronaugh and Maibach, 1999; Sekkat et al., 2004). This approach is able to provide insight into characterization of percutaneous drug permeation and absorption before necessary in vivo testing in living subjects actually takes

* Corresponding author at: Faculty of Pharmacy, University of Manitoba, 50 Sifton Road, Winnipeg, MB R3T 2N2, Canada. Tel.: +1 204 474 6903; fax: +1 204 474 7617.
E-mail address: xgu@cc.umanitoba.ca (X. Gu).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.11.039

place. Using viable biological skin membranes and appropriate experimental conditions, it is also capable of measuring skin metabolism and assessing skin toxicology of various drug candidates (Bronaugh and Maibach, 1999). Nevertheless, difficulty in obtaining ample human skin specimens and the wide variations in skin permeability and sample treatment methods may sometimes hinder data collection and interpretation from in vitro diffusion experiments. For initial transdermal dosage development and evaluation, biological membranes from various animals and certain artificial membranes have been commonly used as surrogate membranes (Hadgraft and Ridout, 1988; Akomeah et al., 2004; Sekkat et al., 2004). They can not only provide satisfactory results that are well correlative of those obtained from using human skin membrane, but also reduce experimental cost and resource that are required with the use of biological human specimens.

In this study, we investigated three artificial membranes for their applicability and suitability in transdermal drug permeation and absorption. Concurrent use of commercially available insect repellent and sunscreen preparations was tested; the transmembrane permeation of the repellent DEET and the sunscreen oxybenzone from different application sequences were evaluated and compared. Results obtained from the study were also statistically correlated with those from using piglet skin in order to determine the similarity or difference in permeation rate and extent among various membrane models.

2. Materials and methods

2.1. Materials

DEET and oxybenzone were purchased from Fluka Chemika GmbH (Buchs, Switzerland) and Riedel-de Haën GmbH (Seelze, Germany), respectively. Acetonitrile, methanol, potassium phosphate monobasic and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Glacial acetic acid and oleyl ether (Brij® 98) were obtained from Mallinckrodt Specialty Chemical Company (Paris, KY, USA) and Sigma–Aldrich Co. (St. Louis, MO, USA), respectively. All solvents were HPLC-grade, while other chemicals were AC-grade. Deionized HPLC-grade water was obtained from a Milli-Q® Pure Water System (Millipore, Nepean, ON, Canada).

Three commercially available insect repellent and sunscreen preparations were purchased from a local pharmacy, and used as obtained without further manipulation. They were OFF!® Skintastic Insect Repellent Spray (7.0% DEET), OFF!® Skintastic Insect Repellent Lotion (7.5% DEET) (S.C. Johnson and Son Ltd., Brantford, ON, Canada), and Coppertone® Sunscreen Lotion (5.0% oxybenzone, SPF 30) (Schering-Plough Healthcare Products, Mississauga, ON, Canada). They were used at different application sequences and proportions in seven diffusion studies; the study codes are listed in Table 1.

2.2. Artificial membranes

Three artificial membranes were selected as the testing models for the diffusion experiments, based on previous reported literatures (Jiang et al., 1998; Stamatialis et al., 2002). Low-density polyethylene (LDPE) membrane was provided as a gift from Key Container (Winnipeg, MB, Canada). It was lipophilic in nature and the membrane thickness was 50 μm. Low fouling composite membrane (LFC, Hydranautics, Oceanside, CA, USA) was made of polysulfone membrane, polyester fabric, and polyamide membrane. A polysulfone membrane was cast on the top of polyester fabric, which was further coated with a thin layer of polyamide membrane. The ultimate membrane was described as a thin film composite that could be visualized as a piece of biological skin with different hydrophilic layers. The membrane thickness used was 150 μm, and it was applied in the diffusion experiments with polyester fabric facing the donor compartment. Mixed cellulose esters (MCE, mixture of cellulose acetate and cellulose nitrate) membrane was purchased from Millipore (Bedford, MA, USA). This cellulose membrane possessed hydrophilic properties and its thickness was 130 μm. Prior to each diffusion study, the membranes were cut into small pieces (2 cm × 2 cm), and soaked in deionized water for 2 h before being mounted to the diffusion cells.

Epidermis from 3-week-old piglets was used as a biological membrane control to compare transmembrane permeation of DEET and oxybenzone. Preparation of piglet epidermis was previously described (Gu et al., 2005). The animal use protocol was approved by the University of Manitoba Fort Garry Campus Protocol Management and Review Committee, and conducted according to current guidelines published by the Canadian Council on Animal Care (CCAC).

2.3. Diffusion studies

All diffusion experiments were conducted in an automatic transdermal system (Logan Instruments Corporation, Somerset, NJ, USA), which was composed of six vertical Franz-style diffusion cells, a circulating water bath, a magnetic stir console and an automatic sampling collector. The surface area available for

Table 1
Study design of in vitro diffusion experiments

Study design	Study code						
	1	2	3	4	5	6	7
Repellent (gram)	1.0 (A)	1.0 (B)	–	0.5 (A)	0.5 (A)	0.5 (A)	0.5 (B)
Sunscreen (gram)	–	–	1.0 (C)	0.5 (C)	0.5 (C)	0.5 (C)	0.5 (C)
Use approach	Control 1	Control 2	Control 3	A: top; C: bottom; no mixing	A: bottom; C: top; no mixing	Prior mixing	Prior mixing

A: OFF!® Repellent Spray, B: OFF!® Repellent Lotion, and C: Coppertone® Sunscreen Lotion.

drug diffusion in the Franz cell was 0.64 cm^2. The applied dose in each study provided an infinite drug amount for the diffusion process. Before the membrane was mounted onto the cell, a very thin layer of vacuum grease was applied to the connection surface of the receptor and donor cell to prevent the testing media from leaking. A preheated phosphate buffer (7.0 ml, pH 7.4, 4% Brij® 98, w/v) was then added to the receptor cell. The system was left for 30 min at 300 rpm to reach temperature equilibrium ($37 \pm 0.05\,^{\circ}C$). The testing sample was placed in the donor cell, maintaining a complete and intimate contact with the membrane surface, and the donor cell were covered with a microscope cover glass to form an occlusive environment.

An aliquot of receptor medium (0.1 ml) was collected hourly for 6 h during the diffusion study, followed by the replenishment of same volume of fresh, preheated receptor medium at each sampling interval. Each diffusion experiment was performed in six replicates. Concentrations of DEET and oxybenzone in the receptor fluid were analyzed with an HPLC assay developed and validated in our laboratory.

2.4. HPLC assay

The HPLC system comprised a Waters® Alliance 2690 Solvent Delivery Module, a 996 Photodiode Array Detector, and a Nova-Pak® C_{18} column (3.9 mm × 150 mm, 4 μm) (Milford, MA, USA). The mobile phase was a mixture of acetonitrile, methanol and water (pH 3.0 with acidic acid) (65:20:15, v/v/v), delivered at a flow rate of 1 ml/min. The detection wavelength was 254 nm for DEET and 287 nm for oxybenzone, respectively. Under these chromatographic conditions, the retention time was 1.49 min for DEET and 1.98 min for oxybenzone, with a detection limit of 5 ng DEET and 20 ng oxybenzone. The collected diffusion samples were directly injected for analysis without further treatment. Concentrations of DEET and oxybenzone in the commercial testing preparations were also determined by the same method; no other ingredients or excipients present in the formulations interfered with the measurement of DEET and oxybenzone. The calibration linearity ($r^2 \geq 0.99$) of DEET and oxybenzone ranged 50–2000 and 8–500 ng, respectively. The concentrations of DEET and oxybenzone in receptor media and the commercial products were calculated based on the average calibration curves ($n = 6$).

2.5. Data analysis

The permeability coefficients (K_p) of DEET and oxybenzone from three artificial membranes were calculated using the empirical diffusion equations derived from the Fick's First Diffusion Law (Crank, 1975),

$$J_s = K_p C_s \quad (1)$$

where J_s is the steady-state diffusion flux, and C_s is the saturated drug concentration. K_p is often used to compare penetration profiles for solutes examined under different conditions and related to the rate of diffusion of a solute within a membrane adjusted for differences in membrane thickness and solute concentration.

The overall permeation percentages of DEET and oxybenzone after 6 h of diffusion were calculated based on the ratio of accumulated permeation amount to the actual application amount of the test products in the donor cell. Statistical analysis was performed using two-way ANOVA and Tukey's test (PC-SAS® 8.02, SAS Institute Inc., Cary, NC, USA). The following statistical analyses of the data were conducted: (a) the overall permeation percentages and permeability coefficients of DEET and oxybenzone among three artificial membranes and the piglet skin epidermis; (b) the overall permeation percentages of DEET and oxybenzone among various application sequences within the same artificial membrane. Differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Permeation of DEET

The overall permeation percentages of DEET across LDPE membrane after 6 h are shown in Fig. 1. The repellent lotion significantly increased DEET permeation by 439% compared to the repellent spray. Mixing the repellent with the sunscreen prior to application also significantly increased DEET permeation across the membrane; permeation of DEET from Study 6 was 300% higher than that from Study 1, while permeation of DEET from Study 7 was 59% higher than that from Study 2.

There was no difference in DEET permeation across LFC membrane between the repellent spray control and the repellent lotion control (Fig. 1). While applying the sunscreen lotion prior to the repellent spray without premixing produced no DEET permeation, which had been previously observed in piglet skin (Gu et al., 2005), there was a slight decrease (8%) in DEET permeation when the repellent spray was applied before the sunscreen lotion. Premixing the repellent spray with the sunscreen lotion resulted in a significant decrease (59%) in DEET permeation across LFC membrane; on the other hand, premixing the repellent lotion with the sunscreen lotion produced a significant increase (134%) in DEET permeation across LFC membrane.

Permeation of DEET across MCE membrane was significantly higher in the repellent lotion than the repellent spray (Fig. 1). Study 5 produced a significant increase in DEET permeation (71%) compared to the spray control. While premixing the repellent spray with the sunscreen lotion yielded a significant increase in DEET permeation (123%) compared to the single spray control, premixing the repellent lotion with the sunscreen lotion resulted in a significant decrease of DEET permeation (35%) compared to the single lotion control.

3.2. Permeation of oxybenzone

The overall permeation percentages of oxybenzone across LDPE membrane after 6 h are shown in Fig. 2. Presence of the repellent preparations all significantly increased the permeation of oxybenzone across the membranes. Applying sunscreen first resulted in a 33% increase in oxybenzone permeation; however, applying the repellent spray first produced a much higher increase in oxybenzone permeation (151%) compared to the sin-

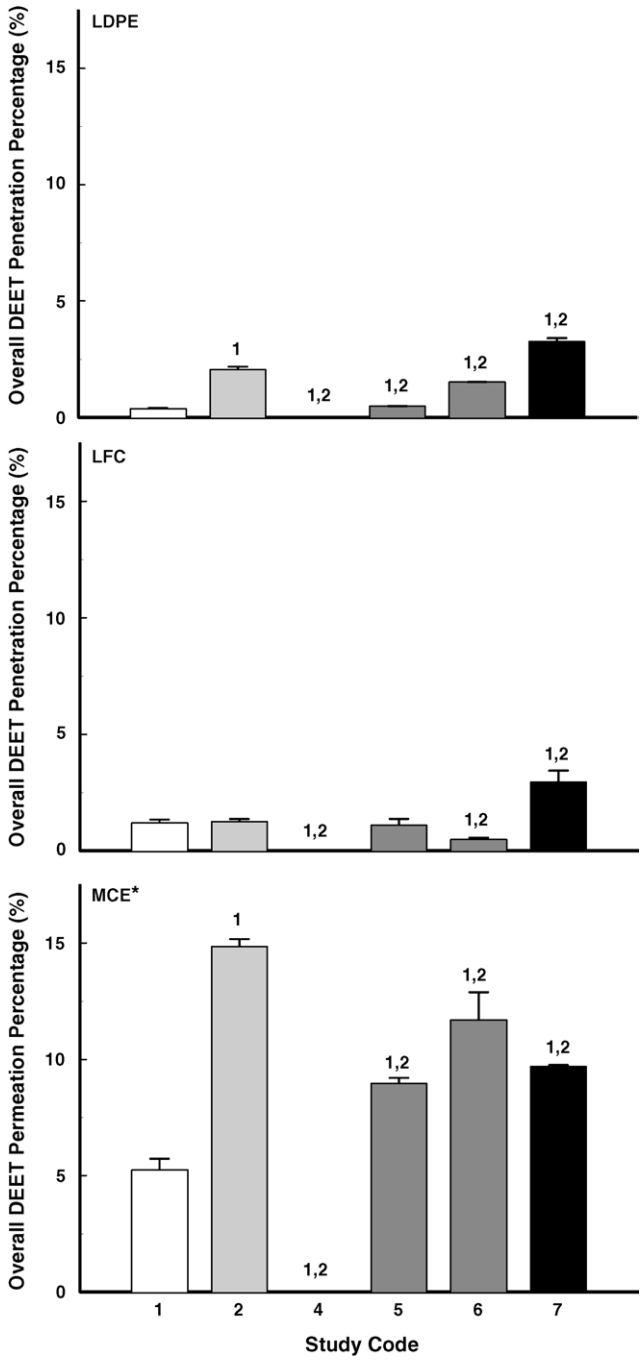

Fig. 1. Overall permeation of DEET across three different artificial membranes after 6 h ($n = 6$, mean ± S.E.M., *significant difference from LDPE and LFC membranes, 1 significant difference from Study 1, 2 significant difference from Study 2, $p \leq 0.05$).

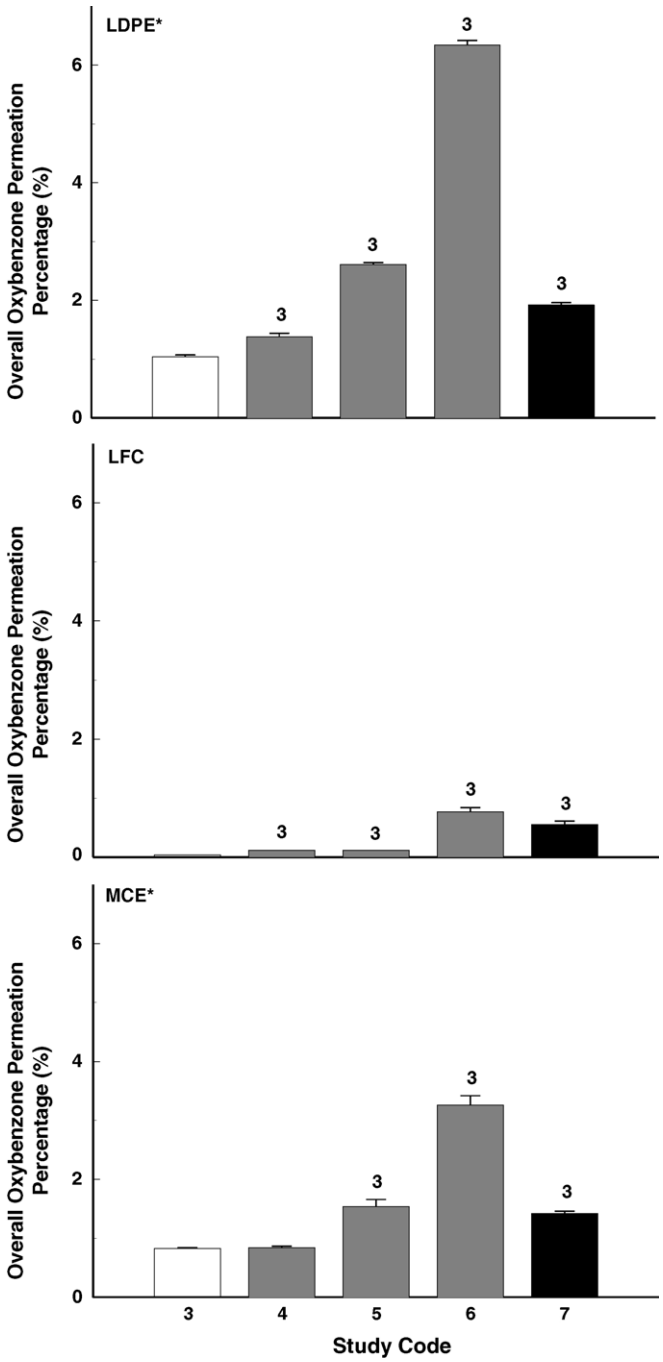

Fig. 2. Overall permeation of oxybenzone across three different artificial membranes after 6 h ($n = 6$, mean ± S.E.M., *significant difference from LFC membrane, 3 significant difference from Study 3, $p \leq 0.05$).

gle sunscreen lotion control. Premixing the sunscreen lotion with the repellent spray also yielded a 510% increase in oxybenzone permeation compared to the control, which clearly indicated an enhancement role of the repellent spray. Premixing both repellent and sunscreen lotions together produced an 84% increase in oxybenzone permeation compared to the sunscreen lotion alone.

The overall permeation of oxybenzone across LFC membrane all increased significantly when DEET was present (Fig. 2). A 200% increase in oxybenzone permeation was observed in both Study 4 and Study 5, in which the sunscreen lotion and the repellent spray were used without premixing. When the sunscreen lotion was premixed with either the repellent spray or the repellent lotion, permeation of oxybenzone was further enhanced by 1825% and 1275%, respectively compared to the single sunscreen control.

For MCE membrane, applying the sunscreen lotion prior to the repellent spray did not result in change in oxybenzone permeation (Fig. 2). However, applying the repellent spray prior to the sunscreen lotion yielded an 83% increase in oxybenzone per-

Table 2
Steady-state permeability coefficient ($\times 10^{-4}$, cm/h) of DEET across various testing membranes

Study membrane	Study code					
	1	2	4	5	6	7
LDPE	1.01 ± 0.15*	11.37 ± 0.72	0.00 ± 0.00	1.89 ± 0.02*	1.59 ± 0.04*	6.32 ± 0.46*
LFC	6.01 ± 0.71*	8.45 ± 0.77*	0.00 ± 0.00	3.70 ± 0.96*	1.17 ± 0.25*	9.43 ± 1.44*
MCE	33.24 ± 1.32*	81.46 ± 1.69*	0.00 ± 0.00	25.14 ± 0.21*	26.11 ± 0.72*	25.35 ± 0.21*
Piglet skin	1.53 ± 0.05	11.98 ± 0.71	0.00 ± 0.00	7.07 ± 1.01	22.06 ± 1.14	45.52 ± 1.76

$n = 6$, mean ± S.E.M.
* Significant difference from piglet skin, $p \leq 0.05$.

Table 3
Steady-state permeability coefficient ($\times 10^{-4}$, cm/h) of oxybenzone across various testing membranes

Study membrane	Study code				
	3	4	5	6	7
LDPE	5.69 ± 0.15*	3.64 ± 0.17*	6.98 ± 0.09*	16.94 ± 0.20*	4.70 ± 0.12*
LFC	0.04 ± 0.01*	0.04 ± 0.00*	0.03 ± 0.00*	2.39 ± 0.23*	1.25 ± 0.20*
MCE	5.91 ± 0.04*	2.87 ± 0.12*	5.86 ± 0.45*	11.94 ± 0.53*	4.59 ± 0.18*
Piglet skin	0.72 ± 0.04	1.36 ± 0.08	0.44 ± 0.03	0.64 ± 0.06	0.33 ± 0.09

$n = 6$, mean ± S.E.M.
* Significant difference from piglet skin, $p \leq 0.05$.

meation. Similarly, premixing the sunscreen with the repellent produced significant enhancement of oxybenzone permeation; 290% increase from the mixture of the sunscreen lotion and the DEET spray and 71% increase from the mixture of the sunscreen lotion and the DEET lotion were observed in Study 6 and Study 7, respectively.

4. Discussion

Human skin specimens from different body sites are believed to affect the transdermal penetration rate and extent of test molecules under a variety of conditions (Feldmann and Maibach, 1967; Scheuplein, 1978). Artificial membranes, on the other hand, are able to minimize diffusion variations and simplify application preparation without the needs for complicated pretreatment procedures and rigorous storage conditions. In addition, the availability of artificial membranes is comparatively accessible and inexpensive. Some success has been found from applying artificial membranes to mimic the percutaneous penetration of drug molecules through the biological membranes (Jiang et al., 1998; Dias et al., 1999; Stamatialis et al., 2002). Specifically, artificial membranes are appropriate for assessing drug release characteristics from a formulation vehicle or a delivery system.

Three artificial membranes with variable physicochemical characteristics were tested in this study. Both lipophilic (LDPE) and hydrophilic (LFC and MCE) membranes were tested for their applicability and suitability in transmembrane characterization of lipophilic molecules DEET and oxybenzone. The permeation of DEET and oxybenzone across another lipophilic membrane poly(dimethylsiloxane) (PDMS) was also studied previously (Gu et al., 2004). By testing these artificial membranes that have also been used in diffusion studies with other drug candidates, we hoped to correlate relationship between the artificial membranes and the biological membranes in order to minimize experimental reliance on the biological specimens and reduce overall study costs in in vitro diffusion experimentation.

Steady-state permeability coefficients of DEET and oxybenzone across these three membranes as well as piglet epidermis control are listed in Tables 2 and 3, respectively. Fig. 3 shows representative diffusion curves of DEET and oxybenzone from three different studies across MCE and LDPE membranes, respectively. Good linearity ($r^2 > 0.99$) was found between the permeation amount and the diffusion time for both compounds across the three test membranes, indicating a shorter time interval to reach steady-state diffusion status and subsequently smaller study variations and better reproducibility than the biological membranes. The overall trend in transmembrane permeation of DEET and oxybenzone from different application sequences was identical to what had been observed in previous studies (Gu et al., 2004, 2005). Applying sunscreen lotion prior to repellent spray without premixing effectively prevented DEET from permeating across the test membranes. Premixing repellent and sunscreen preparations resulted in enhanced permeation of both DEET and oxybenzone. Compared to the piglet skin, transmembrane permeation of DEET was generally reduced in LDPE (ranging 32–84%) and LFC (49–95%) membranes, but increased in MCE (21–838%) membrane. For oxybenzone, permeation decrease was observed only in LFC (17–88%) membrane, both LDPE (56–1148%) and MCE (15–633%) membranes increased the transmembrane permeation. There were statistically significant differences in permeability coefficients of both DEET and oxybenzone among the four membrane models tested, which may have been attributed to different properties of the test compounds and the formulations as well as the lipophilicity and hydrophilicity of the membranes.

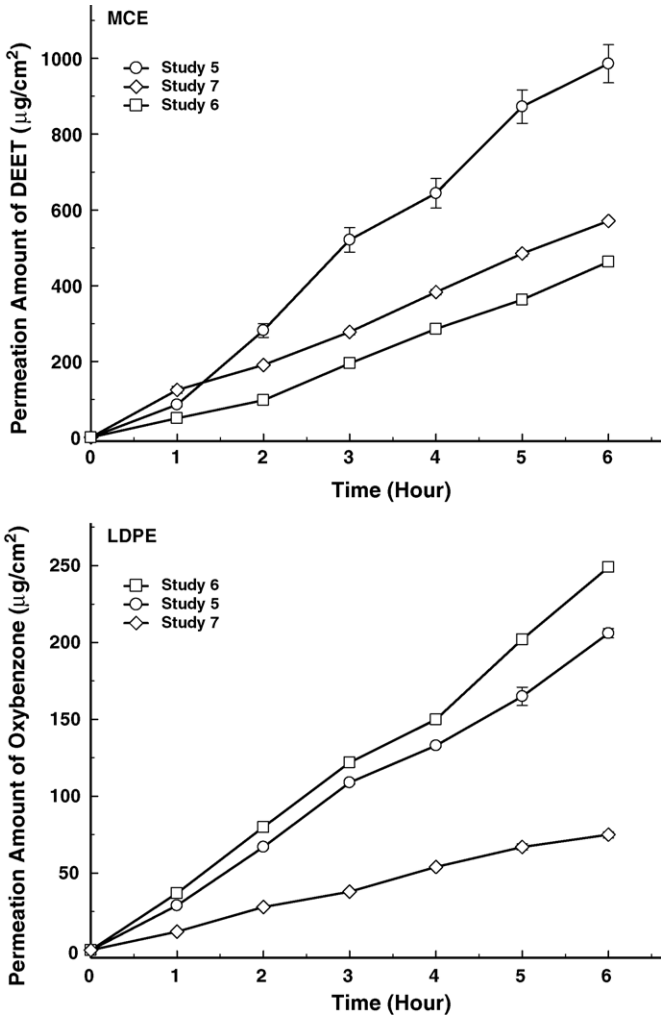

Fig. 3. Representative diffusion curves of DEET and oxybenzone across MCE and LDPE membranes ($n = 6$, mean ± S.E.M., linear correlation $r^2 > 0.99$).

The permeability of DEET and oxybenzone is dependent upon the solubility of DEET and oxybenzone, both in the preparations and in the membranes. DEET and oxybenzone are lipophilic compounds that possess limited solubility in hydrophilic media. Previous studies in our laboratory indicated that permeation of DEET and oxybenzone across lipophilic PDMS membrane in polar solvents such as ethanol and propylene glycol was statistically higher than that in non-polar solvents such as poly(ethylene glycol) 400 (Gu et al., 2004). Solubility of DEET in pH 7.4 phosphate buffer was also significantly higher than that of oxybenzone, which would be indicative of its higher affinity to hydrophilic components than oxybenzone, and subsequently higher permeation percentages across the hydrophilic artificial membranes, LFC and MCE. On the other hand, oxybenzone permeated more through the lipophilic LDPE membrane than the hydrophilic membranes, due mainly to its affinity to lipophilic ingredients. This permeation character of oxybenzone was also previously observed by Jiang et al. The reported values of permeability coefficient of oxybenzone from numerous solvents across high-density polyethylene (HDPE) membrane were all lower than what was found in this study, which was partly attributed to the measurement of single oxybenzone and the use of individual solvent. Commercially available repellent and sunscreen preparations used in this study comprised numerous solvents and additives that could influence transmembrane permeation of oxybenzone. Particularly, the presence of DEET in the study samples could have significantly increased the permeability coefficient of oxybenzone across the test membranes.

Interactions between the test compounds and the artificial membranes could result in variable permeation characteristics of the permeants, which might also be attributed to differences among the tested synthetic membranes and the piglet epidermis. LDPE membrane possessed lipophilic characters in nature, which had a tendency to exclude hydrogen bonding between the diffusion molecules and the polyethylene molecules, rendering its ability to permeate across the membrane. This property would not significantly hinder permeation of DEET and oxybenzone, since both are lipophilic compounds. On the other hand, the solubility capacity of a compound in both organic and aqueous media might influence diffusion and permeation process. The partition coefficients of DEET and oxybenzone in octanol and water ($\log P_{\text{octanol/water}}$) are 2.0 and 3.8, respectively (Cecchine et al., 2000; Balmer et al., 2004), which suggests that oxybenzone possess a higher affinity to lipophilic components than DEET. This property might reflect in the observation of LDPE membrane where the permeation of oxybenzone was increased but that of DEET was reduced compared to the piglet epidermis. For hydrophilic membranes tested in this study, LFC membrane seemed more suitable as a substitute membrane model than MCE membrane, even though the results obtained were generally suppressed compared to the piglet membrane. It was previously tested for the iontophoretic transdermal delivery of timolol maleate, and was found simulating the function of stratum corneum because of its unique three-layer structures (Stamatialis et al., 2002). This property could be used to investigate the permeation profiles of drug delivery across stratum corneum. Human skin is less permeable than most animal skin models according to the literatures (Bronaugh and Maibach, 1999). MCE membrane did not reflect the actual permeation characterization of DEET and oxybenzone, because there were significant differences in several aspects between this membrane and the piglet skin. Its suitability as a diffusion membrane for some other compounds is to be further evaluated in our laboratory. The selection and use of artificial membranes should always be investigated carefully and individually, because data correlation is critical in drug development and delivery. Significant discrepancy between biological and artificial membranes could lead to incorrect experimental results and prediction, which would subsequently potentiate erroneous medical consequences in humans.

The physical properties of the formulation also played an important role in permeability of DEET and oxybenzone across the artificial membranes. Permeation of both DEET and oxybenzone from the mixture of the repellent and sunscreen lotions through the piglet skin produced a higher value than that of the mixture of the repellent spray and the sunscreen lotion (Gu et

al., 2005). This characterization was not observed in the MCE membrane; neither was the case for the permeation of oxybenzone through the LDPE and LFC membranes in Study 7. This might be attributed to differences in viscosity of the mixtures and the partition coefficients of the test compounds. Mixing the repellent spray with the sunscreen lotion introduced more liquid phase to the vehicle, subsequently resulting in lower viscosity and better diffusion environment for the diffusants. Oxybenzone molecules would have better diffusion potentials in less viscous vehicle, and subsequently a higher permeation extent in Study 6 than in Study 7. Previous studies have also indicated that permeation of DEET and oxybenzone was dependent upon formulation types and excipients (Aghazarian et al., 1999; Ross and Shah, 2000; Wissing and Müller, 2002). Permeation of sunscreen filters phenylbenzimidazole and methylbenzylidene camphor from two oily gels was higher than from an oil-in-water emulsion (Aghazarian et al., 1999). The permeation of oxybenzone in this study was in agreement with that observation.

The thickness and pore size of a membrane model might dictate the rate and extent of permeation of a test component. Even though all three artificial membranes tested in the study were thinner than the piglet membrane, significant differences in permeation percentages of DEET and oxybenzone were reflected in permeability coefficients of the two compounds. Biological membranes tend to hydrate when in contact with the receptor medium for an extended period of time. Artificial membranes, on the other hand, do not normally deform due to their special manufacturing process. In addition, formation of drug reservoir in biological membranes from transdermal penetration would unlikely occur in the artificial membranes. Preliminary study in an animal model in our laboratory did find prolonged skin retention of DEET and oxybenzone after topical applications of the preparations. All these factors should be taken into consideration when interpreting permeation data from experiments with artificial membrane models.

5. Conclusion

Percutaneous enhancement of DEET and oxybenzone from concurrent use of commercially available insect repellent and sunscreen products was further observed in three artificial membrane models. There were variations among the test membranes in terms of rate and extent of transmembrane permeation, due mainly to physical and chemical characteristics of the membranes, the test molecules and the formulations. Compared to biological piglet skin, all three artificial membranes produced significant difference in permeability coefficients of the repellent DEET and the sunscreen oxybenzone. However, LDPE and LFC, respectively exhibited permeation patterns of DEET and oxybenzone that were correlative of the data obtained from using piglet skin. Among the three artificial membranes, repellent DEET showed similar permeation extent across lipophilic LDPE and hydrophilic LFC, while sunscreen oxybenzone produced similar permeation extent across lipophilic LDPF and hydrophilic MCE. For in vitro evaluation purposes, artificial membrane models are considered satisfactory candidates for initial diffusion experiments, because they are relatively inexpensive, readily commercially available and batch-to-batch reproducible.

Acknowledgements

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

References

Abdel-Rahman, A., Dechkovskaia, A.M., Goldstein, L.B., Bullman, S.H., Khan, W., El-Masry, E.M., Abou-Donia, M.B., 2004. Neurological deficits induced by malathion, DEET, and permethrin, alone or in combination in adult rats. J. Toxicol. Environ. Health A 67, 331–356.

Aghazarian, V., Tchiakpe, L., Reynier, J.P., Gayte-Sorbier, A., 1999. Release of benzimidazole and benzylidene camphor from topical sunscreen formulations. Drug Dev. Ind. Pharm. 25, 1277–1282.

Akomeah, F., Nazir, T., Martin, G.P., Brown, M.B., 2004. Effect of heat on the percutaneous absorption and skin retention of three model penetrants. Eur. J. Pharm. Sci. 21, 337–345.

Balmer, M.E., Buser, H.R., Müller, M.D., Poiger, T., 2004. Occurrence of the organic UV filter compounds BP-3, 4-MBC, EHMC, and OC in waster water, surface waters, and in fish from Swiss lakes. http://www.faw.ch/wissen_und_Beratung/pflanzenschutz/produktechemie/uv_filter_compou.

Bronaugh, R.L., Maibach, H.I., 1999. Percutaneous Absorption, 3rd ed. Marcel Dekker Inc., New York.

Cecchine, G., Golomb, B.A., Hilborne, L.H., Spektor, D.M., Anthony, C.R., 2000. A review of the scientific literature as it pertains to Gulf War Illnesses, vol. 8: pesticides. National Defense Research Institute, RAND. http://www.gulflink.osd.mil/library/randrep/pesticides_paper/index.html.

Chatelain, E., Gabard, B., Surber, C., 2003. Skin penetration and sun protection factor of five UV filters: effect of the vehicle. Skin Pharmacol. Appl. Skin Physiol. 16, 28–35.

Crank, J., 1975. The Mathematics of Diffusion, 2nd ed. Oxford University Press, New York.

Dias, M., Farinha, A., Faustino, E., Hadgraft, J., Pais, J., Toscano, C., 1999. Topical delivery of caffeine from some commercial formulations. Int. J. Pharm. 182, 41–47.

Feldmann, R.J., Maibach, H.I., 1967. Regional variation in percutaneous penetration of ^{14}C cortisol in man. J. Invest. Dermatol. 48, 181–183.

Felton, L.A., Wiley, C.J., Godwin, D.A., 2004. Influence of cyclodextrin complexation on the in vivo photoprotective effects of oxybenzone. Drug Dev. Ind. Pharm. 30, 95–102.

Fradin, M.S., 1998. Mosquitoes and mosquito repellents: a clinician's guide. Ann. Intern. Med. 128, 931–940.

Gu, X., Kasichayanula, S., Fediuk, D.J., Burczynski, F.J., 2004. In vitro permeation of the insect repellent *N*,*N*-diethyl-*m*-toluamide (DEET) and the sunscreen oxybenzone. J. Pharm. Pharmacol. 56, 621–628.

Gu, X., Wang, T., Collins, D.M., Kasichayanula, S., Burczynski, F.J., 2005. In vitro evaluation of concurrent use of commercially available insect repellent and sunscreen preparations. Br. J. Dermatol. 152, 1263–1267.

Hadgraft, J., Ridout, G., 1988. Development of model membranes for percutaneous absorption measurements. Part 2. Dipalmitoyl phosphatidylcholine, linoleic acid and tetradecane. Int. J. Pharm. 42, 97–104.

Hayden, C.J., Roberts, M.S., Benson, H.A.E., 1997. Systemic absorption of sunscreen after topical application. Lancet 350, 863–864.

Higuchi, W.I., 1967. Diffusional models useful in biopharmaceutics. Drug release rate process. J. Pharm. Sci. 56, 315–324.

Jiang, R., Benson, H.A., Cross, S.E., Roberts, M.S., 1998. In vitro human epidermal and polyethylene membrane penetration and retention of the

sunscreen benzophenone-3 from a range of solvents. Pharm. Res. 15, 1863–1868.

Kasichayanula, S., House, J.D., Wang, T., Gu, X., 2005. Simultaneous analysis of insect repellent DEET, sunscreen oxybenzone and five relevant metabolites by reversed-phase HPLC with UV detection: application to an in vivo study in a piglet model. J. Chromatogr. B 822, 271–277.

Koren, G., Matsui, D., Bailey, B., 2003. DEET-based insect repellents: safety implications for children and pregnant and lactating women. Can. Med. Assoc. J. 169, 209–212.

Montemarano, A.D., Gupta, R.K., Burge, J.R., Klein, K., 1997. Insect repellents and the efficacy of sunscreen. Lancet 349, 1670–1671.

Murphy, M.E., Montemarano, A.D., Debboun, M., Gupta, R., 2000. The effect of sunscreen on the efficacy of insect repellent: a clinical trial. J. Am. Acad. Dermatol. 43, 219–222.

Pont, A.R., Charron, A.R., Brand, R.M., 2004. Active ingredients act as topical penetration enhancers for the herbicide 2,4-dichlorophenoxyacetic acid. Toxicol. Appl. Pharmacol. 195, 348–354.

Qiu, H., Jun, H.W., Tao, J., 1997. Pharmacokinetics of insect repellent *N*,*N*-diethyl-*m*-toluamide in beagle dogs following intravenous and topical routes of administration. J. Pharm. Sci. 86, 514–516.

Ross, J.S., Shah, J.C., 2000. Reduction in skin permeation of *N*,*N*-diethyl-*m*-toluamide (DEET) by altering the skin/vehicle partition coefficient. J. Control. Release 67, 211–221.

Scheuplein, R.J., 1978. The physiology and pathophysiology of the skin. In: Jarrett, A. (Ed.), Site Variations in Diffusion and Permeability. Academic Press, New York, pp. 1731–1752.

Sekkat, N., Kalia, Y.N., Guy, R.H., 2004. Porcine ear skin as a model for the assessment of transdermal drug delivery to premature neonates. Pharm. Res. 21, 1390–1397.

Stamatialis, D.F., Rolevink, H.H.M., Koops, G.H., 2002. Controlled transport of timolol maleate through artificial membranes under passive and iontophoretic conditions. J. Control. Release 81, 335–345.

Staub, D., Debrunner, M., Amsler, L., Steffen, R., 2002. Effectiveness of a repellent containing DEET and EBAAP for preventing tick bites. Wilderness Environ. Med. 13, 12–20.

Wissing, S.A., Müller, R.H., 2002. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. J. Control. Release 81, 225–233.