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# Percutaneous penetration modifiers and formulation effects

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ABSTRACT

The enhancement/retardation of percutaneous permeation of diethyl-*m*-toluamide (DEET) in the presence of five percutaneous penetration modifiers (laurocapram, 3-dodecanoyloxazolidin-2-one (N-0915), *S,S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane (DMBIS), *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane (DMMCBI) and *tert*-butyl 1-dodecyl-2-oxoazepan-3-yl-carbamate (TBDOC)) was investigated. These permeation modifiers were formulated in either water, propylene glycol (PG), ethanol or polyethylene glycol 400 (PEG 400). The permeation studies indicated that laurocapram enhanced DEET permeation in PG, but retarded in PEG 400. Likewise, N-0915 acted as a retardant with ethanol and PEG 400, but not with water. DMBIS decreased the permeation with ethanol as compared to permeation with water, PEG 400 or PG. Similarly, DMMCB acted as a retardant with ethanol and PEG 400, but not with water or PG. TBDOC formulations revealed its activity as a retardant with ethanol, but behaved as enhancer with water, PG and PEG 400. In addition, penetration modifier interactions with stratum corneum ceramide were investigated using chemical modeling. This investigation is significant since it confirms the role of pharmaceutical formulations and shows for the first time that an enhancer can become a retardant or vice versa depending upon the vehicle in which it is applied to the skin. Hence, we should be using the term "penetration modifiers" for all such compounds.



## 1. Introduction

For decades, the utilization of skin as a route for delivering drugs into the body has attracted interest from pharmaceutical formulators. Several physical (Batheja et al., 2007; Lavon and Kost, 2004; Prausnitz, 2004; Wong et al., 2006) and chemical approaches (Asbill et al., 2000) have been explored that compromise the barrier of the uppermost layer of the skin, the stratum corneum. The chemical approach has resulted in the introduction and commercialization of several permeation enhancers ranging from classics such as laurocapram (Hoogstraate et al., 1991; Lewis and Hadgraft, 1990), terpenes (Godwin and Michniak, 1999), alcohols (Andega et al., 2001; Heard and Screen, 2008), glycols (Babu and Pandit, 2005), amides (Fincher et al., 1996; Valenta and Dabic, 2001), sulfoxides (Sarigullu Ozguney et al., 2006), fatty acids (Kandimalla et al., 1999), surfactants (Nokhodchi et al., 2003), etc. to newer compounds such as SEPA009® (Macrochem), NexAct88® (NexMed), SR38® (Pharmetrix) that mainly act by providing fluidity to the lipids of the stratum corneum. Beside the above-mentioned enhancers, a novel subclass of iminosulfuranes has emerged that include compounds such as *S,S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane, that possess enhancement potential (Kim et al., 1999; Strekowski et al., 1999). At the same time that there are efforts in progress to enhance the delivery of therapeutic agents using permeation enhancers, there are increasing concerns over toxicity associated with agrochemicals (Baker et al., 1978), mosquito repellants (Briassoulis et al., 2001), sunscreens (Schlumpf et al., 2001) chemical warfare agents (Schlumpf et al., 2001) and household cleaning chemicals (Mancini, 2004). These concerns have led to the investigation of percutaneous permeation retardants. Permeation retardants, unlike enhancers decrease the diffusion of applied actives by strengthening the intercellular lipid organization of the stratum corneum. Despite the contrasting behavior of enhancers and retardants, they are collectively referred to as "penetration modifiers" since they both act by modifying the structure of stratum corneum (Kaushik et al., 2008) (Purdon, 2005). Even though the concept of percutaneous retardation of permeants is a decade old,

*Abbreviations:* N0915, 3-dodecanoyloxazolidin-2-one; DMBIS, *S,S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane; DMMCBI, *S,S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane; TBDOC, *tert*-butyl 1-dodecyl-2-oxoazepan-3-yl-carbamate; PG, propylene glycol; PEG 400, polyethylene glycol 400; DEET, diethyl-*m*-toluamide; LOQ, limit of quantification; HSD, honestly significantly different.

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there has been scant research performed in the area. The retardants reported in the literature are often structural analogues of potent enhancers. For instance, N-0915 (3-dodecanoyloxazolidin-2-one) is a structural analogue of laurocapram (Hadgraft et al., 1996).

Hadgraft et al. proposed several theories to explain the mechanism of action as enhancers or retardants. It is believed that the action of enhancers and retardants can be explained through their interaction with ceramides (especially ceramide 6) that form the largest group of lipids in the stratum corneum. The ceramides along with cholesterol, fatty acids, cholesterol esters and cholesterol sulfate form multiple lipid lamellae that ultimately provide diffusional resistance to the permeation of the active across the stratum corneum. Among the various ceramides, Hadgraft and coworkers used ceramide 6 to demonstrate the properties of a penetration modifier as an enhancer or retardant. Ceramide 6 was chosen for modeling studies since Wertz (Wertz, 1992) indicated that ceramide 6 possesses the highest hydrogen bonding capability among the various ceramides present in human stratum corneum. Hadgraft reported that one-sided H-bonding of permeation modifiers with ceramide 6 suggests its activity as an enhancer, whereas two-sided interactions imply its role as a retardant (Hadgraft et al., 1996).

The objectives of this current study were to investigate the stratum corneum modifying effects of some relatively potent percutaneous permeation enhancers/retardants including laurocapram, N-0915, *S*,*S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane (DMBIS), *S*,*S*-dimethyl-*N*-(2-methoxycarbonylbenzenesulfonyl) iminosulfurane (DMMCBI) and *tert*-butyl 1-dodecyl-2-oxoazepan-3-yl-carbamate (TBDOC) that were formulated in simple pharmaceutical vehicles. The structures of the penetration modifiers used are depicted in Fig. 1. The solvents used for formulating the penetration enhancers/retardants were water, ethanol, propylene glycol and polyethylene glycol 400 (PEG 400). Diethyl-*m*-toluamide (DEET) a commonly used mosquito repellant was selected as the model permeant for the study. The study also involved evaluation of each penetration modifier as an enhancer or retardant, based on its interaction with ceramide 6 molecules (as suggested by Hadgraft) through modeling. Throughout, we have addressed permeation enhancers and retardants collectively as "penetration modifiers".

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Chemicals

3-dodecanoyloxazolidin-2-one (N-0915), *tert*-butyl 1-dodecyl-2-oxoazepan-3-yl-carbamate (TBDOC) were obtained as generous gifts from Dr. James Chapman from the University of South Carolina, Columbia, SC. Laurocapram, *S*,*S*-dimethyl-*N*-(4-bromobenzoyl) iminosulfurane (DMBIS), *S*,*S*-dimethyl-*N*-(4-methoxycarbonylbenezenesulfonyl) iminosulfurane (DMMCBI) were provided by New Jersey Center for Biomaterials (Piscataway, NJ). Diethyl-*m*-toluamide (DEET), propylene glycol, ethanol were purchased from Sigma Aldrich and polyethylene glycol 400 (PEG 400), phosphate buffer saline tablets were obtained from Fisher Chemicals. All other chemicals used were of analytical grade.

### 2.2. Skin membranes

All skin membranes were purchased from Allosource (Cincinnati, Ohio) and were dermatomed to approximately 380–500 μm and derived from male and female individuals aged between 30 and 60 years. These skin pieces were stored at −80 °C until use, but for no longer than 2 months. Prior to each experiment, the skin samples were thawed and hydrated for 1 h in PBS by mounting on jacketed Franz diffusion cells, which were maintained at 37 °C.

### 2.3. Methods

#### 2.3.1. Preparation of formulations

Penetration modifiers (laurocapram, N-0915, TBDOC, DMBIS, DMMCBI) were weighed and each was added to one of the four vehicles (water, propylene glycol, ethanol and PEG 400) to prepare 0.4 M solutions or suspensions. The solution/suspension was then vortexed at room temperature for 48 h. The solution/suspension obtained was centrifuged at 8000 rpm for 5 min and the supernatant was collected, filtered and used as the final formulation for in vitro skin permeation experiments. The solubility of each penetration modifier in each vehicle was determined (methods described below).

#### 2.3.2. Determination of solubility and available amounts of penetration modifiers in vehicles

Assays for the five penetration modifiers namely laurocapram, N-0915, DMBIS, DMMCBI and TBDOC were developed using HPLC with ultraviolet detection (HP 1100, Agilent Technologies, Inc.). The content of each of penetration modifier in water, propylene glycol, ethanol and PEG 400 was determined. Briefly, this involved taking 0.4 M of each of the penetration modifiers in 100 μl of each of the four solvents followed by vortexing at 32 °C for 48 h. The saturated solution was then centrifuged and supernatant obtained was filtered by Vivaspin 500™ ultrafiltration centrifugal device (Sartorius Biotech., USA), diluted by mobile phase and injected into HPLC and analyzed.

#### 2.3.3. Modeling and partition coefficient determination of penetration modifiers

The molecular modeling of penetration modifiers and ceramide 6 molecules were performed using MOE 2008.09 (Molecular Operating Environment) software from Chemical Computing Group, Toronto Canada. Both the ceramide 6 molecules and penetration modifiers were built using MOE. Their three dimensional (3D) structures were minimized using molecular mechanics force field, MMFF94 (Halgren, 1996). Next, the 3D structures of ceramide 6 were kept rigid and the penetration modifiers were brought in close proximity of the ceramide 6 molecules to mimic possible H-bond formations. The maximum distance between donor-acceptor pairs is less than 3.5 Å to allow the H-bond formation. The pictures shown in Table 3 were generated using the same MOE software package.

The partition coefficients ($A \log P$) of each penetration modifier were calculated using Pipeline Pilot software package (Accelrys Software Inc., San Diego, CA). The $A \log P$ component of Pipline Pilot was used to calculate the Ghose/Crippen group-contribution estimate for $\log P$ (Ghose et al., 1998), where $P$ is the relative solubility of a compound in octanol vs. water.

#### 2.3.4. In vitro skin permeation study

Human cadaver skin (Allosource, Cincinnati, OH) prehydrated with phosphate buffered saline, pH 7.4 was mounted on vertical Franz diffusion cells (Permegear, Inc., Bethlehem, PA). The in vitro skin permeation studies were performed using Franz diffusion cells with receptor compartment of 5.1 ml volume and donor compartment of 1 ml capacity. Each skin piece was treated with 50 μl of selected formulations containing laurocapram or N-0915 prepared in ethanol, water, propylene glycol or PEG 400 1 h prior to application of the permeant, DEET. This was followed by 100 μl application of neat solution of DEET (infinite dose approach) over a surface area of 0.64 $cm^2$. DEET was chosen as the model permeant. Similarly, skin pieces were treated with 50 μl of DMBIS, DMMCBI and TBDOC prepared in ethanol, water, propylene glycol or PEG 400 1 h

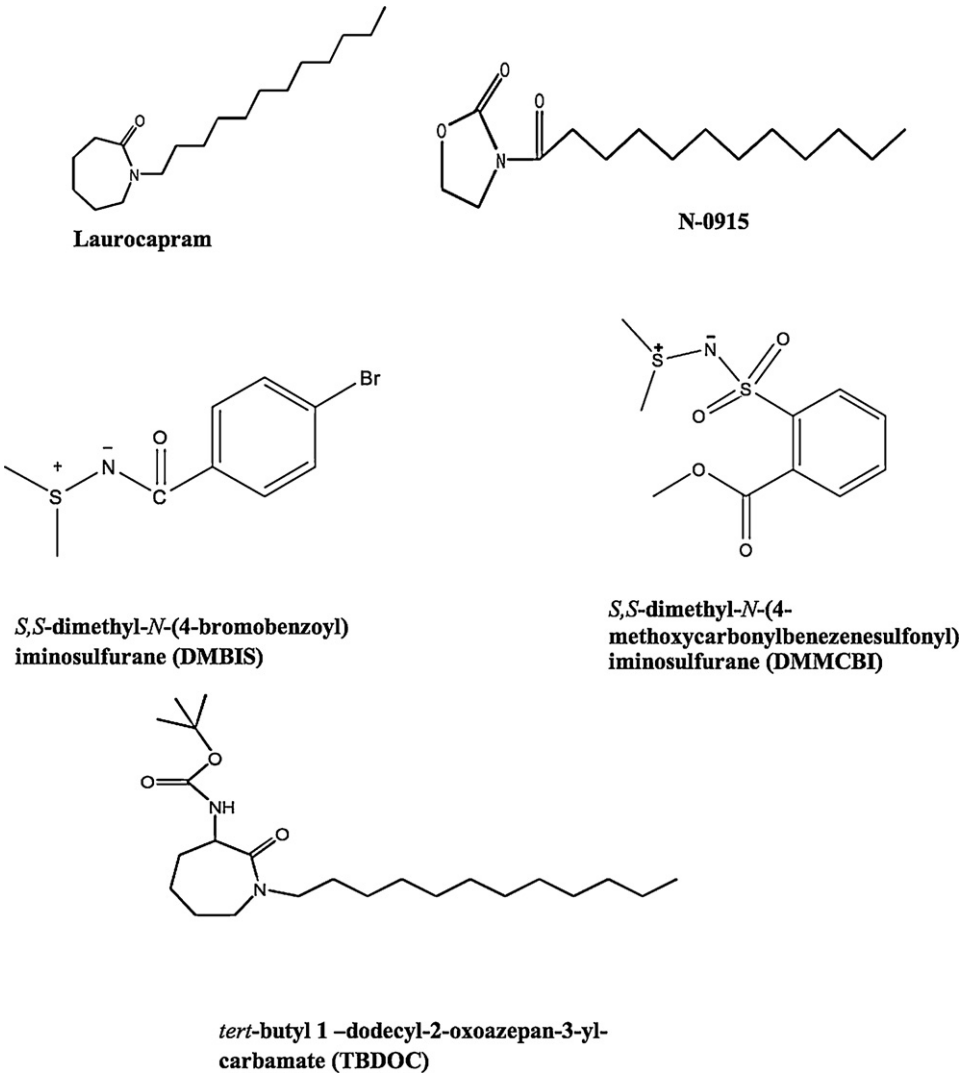



**Fig. 1.** Structures of penetration modifiers.

prior to application of 100 μl of neat DEET. The donor compartment was covered with Parafilm® to minimize the evaporation of the formulation of penetration modifier or the permeant. The receptor compartment stirred at 600 rpm contained phosphate buffered saline at pH 7.4 and was maintained at 37 °C using thermostatic water pump (Haake DC10, Karlsruhe, Germany). 300 μl aliquots were withdrawn from the receptor at 0, 2, 4, 6, 8, 23, 24, 26, 28 and 30 h and replaced by an equivalent amount of fresh phosphate buffer. Suitable controls were also run in the study, and greater than three replicates were run for each formulation. These controls included no treatment, treatment with laurocapram alone, water alone, propylene glycol alone, ethanol alone and PEG 400 alone followed by application of DEET on the skin surface.

The experimental data (drug concentration values) were corrected for progressive dilution using the following equation:

$$M_t(n) = V_r C_n + V_s \sum C_m$$

where $M_t(n)$ is the cumulative mass of the drug transported across the skin membrane at time $t$, $C_n$ represents drug concentration in the receiver compartment and $V_r$ corresponds to volume of the receptor compartment; $\Sigma C_m$ refers the summed total of the previous measured concentrations [$m = 1$ to $(n-1)$] and $V_s$ denotes to the volume of the sample removed for analysis (Meiden et al., 2003).

All in vitro skin permeation experiments were performed with human cadaver skin obtained from three different donors. The percutaneous permeation parameters obtained in case of each formulation from each piece of skin was then examined for reproducibility on repetition of the same experiment using skin piece derived from different donor. This was the method used for ensuring the integrity of the skin during the experiment.

### 2.3.5. Analysis of DEET and penetration modifiers

The analysis of DEET and penetration modifiers was performed using HPLC (HP 1100, Agilent Technologies, Inc.) equipped with degasser (G1379A), autosampler (G1313A), quaternary pump (G1311A), a UV–vis diode array (G1315A) and an Eclipse XDB-C18 RP column (Agilent Technologies, USA) having a pore size of 5 μm and dimensions equivalent to 4.6 mm × 150 mm. The details of HPLC methods of DEET and penetration modifiers are listed in Table 1. An external standard technique was employed for all the test compounds.

All methods were validated for linearity, precision and accuracy. The correlation coefficients of 0.999 for linearity of plots were observed in case of DEET and all penetration modifiers used in the study. Intraday variability was less than 0.2% for all methods, and interday variability was also calculated to be less than 3.0% for all penetration modifiers.

**Table 1**
HPLC methods of DEET and penetration modifiers.

| Active/penetration modifier | Mobile phase | Flow rate (ml/min), column temperature (°C) | Injection volume (μl) | Detection wavelength (nm), retention time (min) | Limit of quantification (LOQ) (μg/ml) |
|---|---|---|---|---|---|
| DEET | Methanol:water (80:20, v/v) | 0.7, 25 | 20 | 240, 3.4 | 1.9 |
| Laurocapram | Acetonitrile:methanol:water (88:2:10, v/v/v) | 1.5, 40 | 25 | 210, 4.4 | 0.03 |
| N-0915 | Acetonitrile:water (80:20, v/v) | 1.5, 40 | 25 | 210, 4.2 | 0.14 |
| DMBIS | Acetonitrile:methanol:water (65:15:35, v/v/v) | 1, 40 | 20 | 245, 3.9 | 0.12 |
| DMMCBI | Acetonitrile:methanol:ammonium phosphate buffer (20:5:75, v/v/v), pH 3.0 | 1, 40 | 10 | 270, 2.9 | 4.72 |
| TBDOC | Acetonitrile:water (65:5, v/v) | 1.5, 40 | 25 | 210, 4.9 | 0.79 |

### 2.4. Data and statistical analysis

The amount of active present in the samples was determined using validated assay methods. In vitro permeation studies, several transdermal parameters such as mean flux, cumulative amount permeated after 30 h and permeability coefficient were calculated. For determination of the mean flux, cumulative amounts of permeant per area (μg/cm$^2$) was plotted against time (h) and flux was calculated as the slope of the linear portion of the plot (between 8 and 30 h) using linear regression (Microsoft Excel) (Batheja et al., 2009). The permeability coefficient (cm/h) was calculated from the ratio of mean flux and permeant concentration (μg/ml) in the donor compartment.

The degree of enhancement/retardation was evaluated from the modifier ratio, MR, which was calculated as described below:

$MR_J$ = $J$ in the presence of treatment/$J$ in the absence of treatment.
$MR_{Q_{30}} = Q_{30}$ in the presence of treatment/$Q_{30}$ in the absence of treatment.

The vehicles used in the study themselves act as penetration modifiers. Therefore DEET permeation was determined in the presence of the penetration modifier and was compared with DEET permeation in the presence of vehicles alone using MR* values:

$MR^*_J$ = $J$ in the presence of penetration modifier-vehicle formulation/$J$ in the presence of vehicle alone.
$MR*_{Q_{30}} = Q_{30}$ in the presence of penetration modifier-vehicle formulation/$Q_{30}$ in the presence of vehicle alone.

The values above unity represented enhancement and values below 1 represented retardation of the permeant.

All results were statistically analyzed using Minitab software version 15 (State College, PA) with multiple comparison tests done using Tukey HSD method.

## 3. Results and discussion

### 3.1. Determination of solubility and available amounts of penetration modifiers in vehicles

Content of the five penetration modifiers (laurocapram, N-0915, DMBIS, DMMCBI and TBDOC) was determined in water, propylene glycol, ethanol and PEG 400. The solubility in mg/ml is depicted in Table 2. It is a well-known fact that laurocapram is insoluble in water, however, a detectable amount of laurocapram was recorded in our laurocapram–water formulation. This is probably due to the fact that our study involved formulation of a non-emulsifier stabilized emulsion of laurocapram and water. Laurocapram content determination in other laurocapram formulations such as laurocapram–PG and laurocapram–PEG 400 showed a similar degree of solubility ($p > 0.05$). However, relatively less laurocapram was observed in the laurocapram–ethanol formulation. N-0915 was undetected in N-0915–water formulation, but some N-0915 was detected in both ethanol and propylene glycol. The highest amount of N-0915 was obtained in PEG 400. On the other hand, DMBIS showed little solubility in water but recordable amounts were observed in propylene glycol, ethanol and PEG 400. In contrast, DMMCBI dissolved in water, propylene glycol and PEG 400, but had better solubility in ethanol ($p < 0.05$). TBDOC showed no solubility in water but dissolved to some extent in propylene glycol, ethanol and PEG 400 ($p > 0.05$).

### 3.2. Modeling and partition coefficient determination of penetration modifiers

Software generated partition coefficients showed relatively high lipophilicity of laurocapram, N-0915 and TBDOC, justifying their low or non-detectable solubility in water. The results also provided information concerning the relative hydrophilic nature of DMMCBI and DMBIS that is supported by the higher solubility of DMBIS and DMMCBI in water.

Each penetration modifier selected for this study was subjected to modeling studies to examine its potential as an enhancer or retardant. Hadgraft based the definition of enhancers and retardants on the way the compound interacted with ceramide 6. We used the same criterion in the study (Hadgraft et al., 1996).

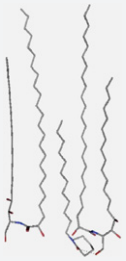
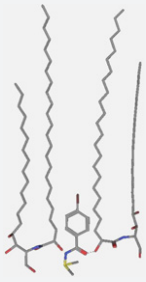
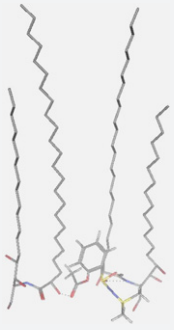
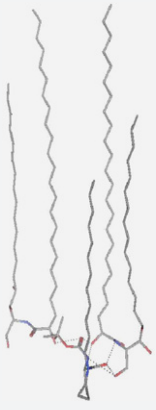
The interactions of ceramide 6 with penetration modifiers obtained from modeling studies, as well as partition coefficients, are listed in Table 3. Modeling studies revealed that laurocapram and DMBIS are capable of forming one-sided H-bonding; a finding which suggests that they are potential enhancers, based on Hadgraft's proposed theory. It has been hypothesized (Hadgraft et al., 1996) that one-sided H-bonding of enhancers with skin lipids especially ceramides disturbs the inter H-bonding among the ceramide molecules leading to fluidization of the stratum corneum, causing enhancement in active permeation. Both laurocapram (Hoogstraate et al., 1991) and DMBIS (Kim et al., 1999) have been reported to

**Table 2**
Determination of solubility and available amounts of penetration modifiers in vehicles.

| Penetration modifier | Solubility (mg/ml) ± SD ($n$ = 3) | | | |
|---|---|---|---|---|
| | Water[a] | Propylene glycol[a] | Ethanol[a] | PEG 400[a] |
| Laurocapram | 16.9 ± 4.0 | 104.4 ± 0.5 | 64.4 ± 5.3 | 109.9 ± 7.8 |
| N-0915 | Below LOQ | 5.76 ± 0.6 | 0.7 ± 0.01 | 25.8 ± 9.2 |
| DMBIS | 2.7 ± 1.0 | 26.7 ± 4.46 | 45.30 ± 2.8 | 47.9 ± 12.8 |
| DMMCBI | 48.2 ± 1.3 | 27.6 ± 4.0 | 119.6 ± 10.1 | 43.8 ± 10.5 |
| TBDOC | Below LOQ | 43.5 ± 2.7 | 17.8 ± 2.5 | 34.1 ± 3.8 |

SD represents standard deviation.
[a] Solvent.

**Table 3**
Partition coefficient of penetration modifiers and computer generated potential interactions of ceramide 6 molecules with penetration modifiers.

| Penetration modifier | Penetration modifier ceramide 6 molecules | Log *P* |
|---|---|---|
| Laurocapram |  | 4.6 |
| N-0915 |  | 5.3 |
| DMBIS |  | 2.6 |
| DMMCBI |  | 1.3 |
| TBDOC |  | 6.2 |

The colored annotations are representation of certain atoms modeling software, where red represents oxygen, yellow indicates sulfur, grey refers to carbons, blue represents nitrogen, maroon indicates bromine and black dots represents possible H-bonds.

enhance the permeation of numerous actives; a finding that agrees with results obtained from modeling studies. However, N-0915, DMMCBI and TBDOC are capable of forming multiple two-sided H-bonds with ceramide 6 suggesting retardation behavior. The percutaneous retardation activity of N-0915 and DMMCBI has been confirmed by reports from Hadgraft (Hadgraft et al., 1996) and Song (Song et al., 2005) on permeation of DEET and hydrocortisone respectively. Also reports by Purdon indicate TBDOC to be potential retardant of actives (Purdon, 2005).

### 3.3. In vitro skin permeation study results

In order to investigate the effect of the modifier compounds in various vehicles, in vitro percutaneous permeation studies were conducted using DEET as the model compound. The penetration modifiers selected were either nitrogen or sulfur containing compounds and included laurocapram, N-0915, DMBIS, DMMCBI and TBDOC. The study involved penetration modifiers that have been reported to act as enhancers (Hoogstraate et al., 1991; Sintov et al., 2009) and also included some compounds that have shown retardation activity for certain actives (Hadgraft et al., 1996; Kim et al., 1999; Purdon, 2005). Solvents used in the study included water, ethanol, PG and PEG 400 that are not only commonly used vehicles in dermal formulations, but some are also good penetration modifiers (Williams and Barry, 2006). DEET was chosen as the permeant since it is a liquid under ambient conditions and in this way we avoided the added complication of other vehicles and solubility issues.

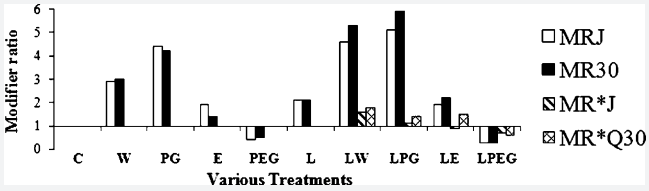
The transdermal permeation parameters such as mean flux between 8 and 30 h, $J$ ($\mu g/cm^2/h$), cumulative amount of DEET permeated after 30 h, $Q_{30}$ and permeability coefficient, $K_p$ (cm/h) were determined. The determination of lag times was not performed because steady state flux of permeant was not achieved in various cases. Therefore mean flux of the permeant was calculated between 8 and 30 h.

#### 3.3.1. Effect of various formulations of laurocapram on permeation of DEET

The permeation parameters for DEET in the presence of laurocapram are summarized in Table 4. It was observed that laurocapram formulated in water, PG or ethanol, enhanced the permeation of DEET by approximately 5, 6 and 2 fold respectively (as indicated by their $MR_{30}$, $MR_J$ values in Table 4). However, laurocapram formulated in PEG 400, resulted in the retardation of DEET with $MR_J$, $MR_{Q_{30}}$ value of 0.3. All treatments containing laurocapram were statistically different as compared to no treatment ($p < 0.05$). $MR^*_J$ and $MR*_{Q_{30}}$ values were also determined to compare the effect of laurocapram formulations with that of treatment with vehicle alone. The cumulative amount of DEET permeated in the presence of various laurocapram formulations is depicted in Fig. 2. It was observed that in the treatment with laurocapram–water, the major contribution to enhancement was due to water, because there was no statistical difference between laurocapram–water and water treatments ($p > 0.05$). However, permeability coefficient, flux and $Q_{30}$ values with laurocapram–water were significantly greater than that after laurocapram treatment ($p < 0.05$). Since our study involved pretreatment of SC with an emulsion (not stabilized with an emulsifier) of laurocapram and water, it seems that laurocapram and water contributed independently towards enhancement of DEET permeation. In laurocapram–PG treatment, no statistical difference in terms of DEET permeability coefficient, flux and $Q_{30}$ values was observed between the laurocapram treatment and PG treatments ($p > 0.05$). However, significant statistical difference was obtained between laurocapram and laurocapram–PG treatments ($p < 0.05$). This suggests that the addition of PG in laurocapram–PG

**Table 4**
Permeation parameters of DEET in the presence of laurocapram in selected vehicles.

| Parameters formulation ($n=5$) | Permeability coefficient, $K_p \times 10^{-5} \pm$ SD (cm/h) | Mean flux, $J \pm$ SD ($\mu g/cm^2/h$) | Cumulative amount of DEET after 30 h, $Q_{30} \pm$ SD ($\mu g/cm^2$) | [a]$MR_J$ | [b]$MR_{30}$ | [c]$MR^*_J$ | [d]$MR^*_{Q_{30}}$ |
|---|---|---|---|---|---|---|---|
| No treatment, C | 6.0 ± 2.0 | 58.0 ± 19.4 | 1413.9 ± 315.6 | 1.0 | 1.0 | – | – |
| Water, W | 17.3 ± 2.36 | 168.2 ± 22.9 | 4243.4 ± 364.2 | 2.9 | 3.0 | 1.0 | 1.0 |
| Propylene glycol, PG | 26.4 ± 6.4 | 256.0 ± 61.7 | 5894.6 ± 1458.6 | 4.4 | 4.2 | 1.0 | 1.0 |
| Ethanol, E | 11.6 ± 2.4 | 112.6 ± 23.2 | 2040.5 ± 1266.3 | 1.9 | 1.4 | 1.0 | 1.0 |
| PEG 400, PEG | 2.6 ± 1.2 | 25.6 ± 12.2 | 701.7 ± 312.0 | 0.4 | 0.5 | 1.0 | 1.0 |
| Laurocapram | 12.6 ± 2.8 | 122.3 ± 26.8 | 2984.3 ± 741.3 | 2.1 | 2.1 | – | – |
| Laurocapram in water, LW | 27.7 ± 3.8 | 268.3 ± 37.4 | 7546.6 ± 1161.1 | 4.6 | 5.3 | 1.6 | 1.8 |
| Laurocapram in PG, LPG | 30.0 ± 1.9 | 291.2 ± 18.1 | 8310.2 ± 669.2 | 5.1 | 5.9 | 1.1 | 1.4 |
| Laurocapram in ethanol, LE | 11.5 ± 0.8 | 111.5 ± 7.9 | 3060.4 ± 130.4 | 1.9 | 2.2 | 0.9 | 1.5 |
| Laurocapram in PEG 400, LPEG | 1.9 ± 0.5 | 18.4 ± 4.9 | 478.2 ± 118.9 | 0.3 | 0.3 | 0.7 | 0.6 |




SD represents standard deviation.
[a] Ratio of $J$ of DEET in the presence of treatment to $J$ of DEET in the absence of treatment.
[b] Ratio of $Q_{30}$ of DEET in the presence of treatment to ratio of $Q_{30}$ of DEET in the absence of any treatment.
[c] Ratio of $J$ of DEET in the presence of penetration modifier formulation in a solvent to $J$ of DEET in the presence of solvent alone.
[d] Ratio of $Q_{30}$ of DEET in the presence of penetration modifier formulation in a solvent to $Q_{30}$ of DEET in the presence of a solvent alone.

formulation leads to enhancement of DEET permeation. This finding was not surprising because synergism between laurocapram and PG in permeation of actives has been reported previously (Williams and Barry, 2004). The permeability coefficient, flux and $Q_{30}$ values after laurocapram–ethanol treatment were similar to ethanol and laurocapram treatments. This finding suggests enhancement was due to the presence of ethanol and laurocapram in the formulation ($p > 0.05$). Similarly permeability coefficient, flux and $Q_{30}$ values after laurocapram–PEG 400 and PEG 400 were similar ($p > 0.05$) and retardation was probably due to activity of PEG 400. Similar results were reported by Wotton et al. who reported extremely slow in vitro permeation of metronidazole across full thickness human skin after application of formulation containing 18 μmol metronidazole dissolved in vehicles containing 1% V/V of laurocapram and 18% W/V PEG 400 (Wotton et al., 1985). The authors attributed this observation to no release of metronidazole from the vehicle containing laurocapram and PEG 400.

### 3.3.2. *Effect of N-0915 in selected vehicles on permeation of DEET*

The permeation parameters for DEET after application of N-0915 in selected vehicles are listed in Table 5. All N-0915 treatments were significantly different from controls (no treatment) in terms of permeability coefficient, flux and $Q_{30}$ values ($p < 0.05$) except N-0915–PG ($p > 0.05$). When the effect of formulations of N-0915 were compared with controls on permeation of DEET, it was observed that N-0915–water enhanced the permeation of DEET, whereas N-0915–PG, N-0915–ethanol, N-0915–PEG 400 retarded the permeation (Fig. 3). The enhancement of DEET in the presence of N-0915–water was probably caused by the low solubility of N-0915 in water. This is evident by $MR^*_J$ and $MR*_{Q_{30}}$ values close to unity for N0915–water and similar flux and $Q_{30}$ values of DEET in the presence of N-0915–water and water alone ($p > 0.05$). But it does not imply that enhancement of DEET in the presence of N-0915–water formulation can be entirely attributed to the presence of water in the formulation. Even though, N-0915 was undetected by our analytical technique in the N-0915–water

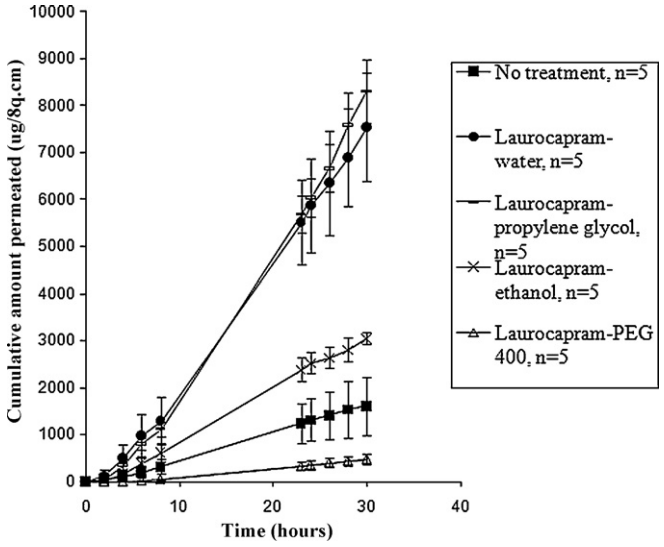



**Fig. 2.** Permeation profile of DEET in the presence of laurocapram formulations.

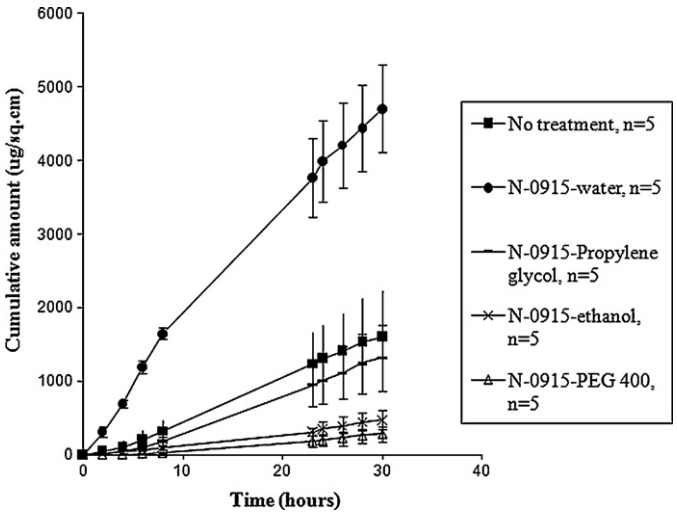
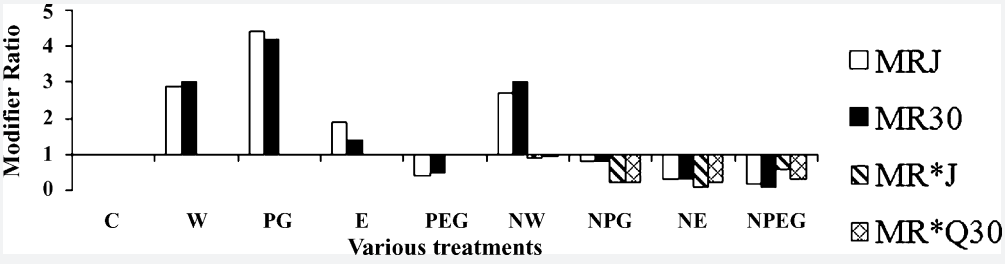



**Fig. 3.** Permeation profile of DEET in the presence of N-0915 formulations.

**Table 5**
Permeation parameters of DEET in the presence of N-0915 in selected vehicles.

| Parameters formulation ($n = 5$) | Permeability coefficient, $K_p \times 10^{-5} \pm$ SD (cm/h) | Mean flux, $J \pm$ SD ($\mu g/cm^2/h$) | Cumulative amount of DEET after 30 h, $Q_{30} \pm$ SD ($\mu g/cm^2$) | [a] $MR_J$ | [b] $MR_{30}$ | [c] $MR^*_J$ | [d] $MR^*_{Q30}$ |
|---|---|---|---|---|---|---|---|
| No treatment | 6.0 ± 2.0 | 58.0 ± 19.4 | 1413.9 ± 315.6 | 1.0 | 1.0 | – | – |
| Water | 17.3 ± 2.36 | 168.2 ± 22.9 | 4243.4 ± 364.2 | 2.9 | 3.0 | 1.0 | 1.0 |
| PG | 26.4 ± 6.4 | 256.0 ± 61.7 | 5894.6 ± 1458.6 | 4.4 | 4.2 | 1.0 | 1.0 |
| Ethanol | 11.6 ± 2.4 | 112.6 ± 23.2 | 2040.5 ± 1266.3 | 1.9 | 1.4 | 1.0 | 1.0 |
| PEG 400 | 2.6 ± 1.2 | 25.6 ± 12.2 | 701.7 ± 312.0 | 0.4 | 0.5 | 1.0 | 1.0 |
| N-0915 in water | 15.9 ± 2.9 | 154.4 ± 28.6 | 4211.9 ± 573.7 | 2.7 | 3.0 | 0.9 | 1.0 |
| N-0915 in PG | 5.0 ± 1.6 | 48.8 ± 15.8 | 1116.7 ± 354.8 | 0.8 | 0.8 | 0.2 | 0.2 |
| N-0915 in ethanol | 1.7 ± 0.4 | 16.3 ± 4.1 | 402.8 ± 112.3 | 0.3 | 0.3 | 0.1 | 0.2 |
| N-0915 in PEG 400 | 1.5 ± 0.5 | 14.7 ± 4.7 | 224.4 ± 91.7 | 0.2 | 0.1 | 0.6 | 0.3 |

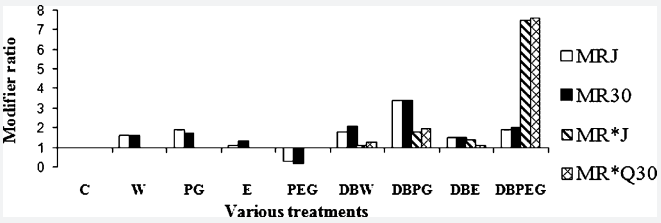



SD represents standard deviation.
[a] Ratio of $J$ of DEET in the presence of treatment to $J$ of DEET in the absence of treatment.
[b] Ratio of $Q_{30}$ of DEET in the presence of treatment to ratio of $Q_{30}$ of DEET in the absence of any treatment.
[c] Ratio of $J$ of DEET in the presence of penetration modifier formulation in a solvent to $J$ of DEET in the presence of solvent alone.
[d] Ratio of $Q_{30}$ of DEET in the presence of penetration modifier formulation in a solvent to $Q_{30}$ of DEET in the presence of a solvent alone.

formulation, there is a possibility that trace amounts of N-0915 were present. These miniscule amounts of N-0915 could have partitioned into the SC lipids via the lipids present on the surface of SC (owing to high log $P$ of N-0915) and in the presence of water in the formulation, causing fluidization/disruption of lipid barrier of the SC leading to enhancement of DEET. The permeability coefficient, flux and $Q_{30}$ values of DEET in the presence of N-0915–PG showed significant decrease in DEET permeation as compared to PG alone treatment ($p < 0.05$). Since N-0915–PG contains N-0915 (a potent retardant) and PG (an enhancer), the resulting antagonistic action of the penetration modifiers resulted in weakening of the retardation activity of N-0915 that was similar to control ($p > 0.05$). Similarly N-0915–ethanol showed a significant decrease in permeability coefficient, flux and $Q_{30}$ values as compared to PG alone ($p < 0.05$). Unlike N-0915–PG, N-0915–ethanol was significantly different from no treatment because the rapid evaporation of ethanol after addition on application site led to a high concentration gradient for rapid diffusion of N-0915 across the stratum corneum (Ibrahim and Li, 2009). As there was little ethanol left at the site of application, its enhancement action was reduced, and there was less effect on the action of N-0915. Comparing the permeability coefficient, flux and $Q_{30}$ values obtained after treatment

**Table 6**
Permeation parameters of DEET in the presence of DMBIS in selected vehicles.

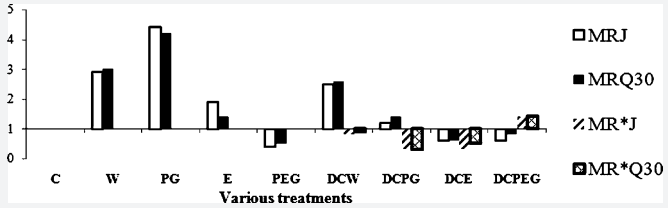
| Parameters formulation ($n = 5$) | Permeability coefficient, $K_p \times 10^{-5} \pm$ SD (cm/h) | Mean flux, $J \pm$ SD ($\mu g/cm^2/h$) | Cumulative amount of DEET after 30 h, $Q_{30} \pm$ SD ($\mu g/cm^2$) | [a] $MR_J$ | [b] $MR_{30}$ | [c] $MR^*_J$ | [d] $MR^*_{Q30}$ |
|---|---|---|---|---|---|---|---|
| No treatment, C | 10.1 ± 2.9 | 97.8 ± 27.8 | 2736.9 ± 695.0 | 1.0 | 1.0 | – | – |
| Water, W | 15.9 ± 1.9 | 154.5 ± 18.7 | 4513.1 ± 671.4 | 1.6 | 1.6 | 1.0 | 1.0 |
| Propylene glycol, PG | 18.9 ± 3.4 | 183.7 ± 33.4 | 4590.7 ± 728.5 | 1.9 | 1.7 | 1.0 | 1.0 |
| Ethanol, E | 11.3 ± 4.6 | 109.8 ± 44.9 | 3667.5 ± 1120.9 | 1.1 | 1.3 | 1.0 | 1.0 |
| PEG 400, PEG | 2.6 ± 1.2 | 25.6 ± 12.2 | 701.7 ± 312.0 | 0.3 | 0.2 | 1.0 | 1.0 |
| DMBIS in water, DBW | 18.4 ± 0.7 | 178.0 ± 7.1 | 5707.5 ± 503.6 | 1.8 | 2.1 | 1.1 | 1.3 |
| DMBIS in PG, DBPG | 34.5 ± 2.3 | 335.0 ± 22.0 | 9414.0 ± 896.8 | 3.4 | 3.4 | 1.8 | 2.0 |
| DMBIS in ethanol, DBE | 15.5 ± 1.5 | 150.1 ± 14.9 | 4232.3 ± 1.5 | 1.5 | 1.5 | 1.4 | 1.1 |
| DMBIS in PEG 400, DBPEG | 19.9 ± 0.8 | 192.8 ± 8.2 | 5344.5 ± 349.8 | 1.9 | 2.0 | 7.5 | 7.6 |




SD represents standard deviation.
[a] Ratio of $J$ of DEET in the presence of treatment to $J$ of DEET in the absence of treatment.
[b] Ratio of $Q_{30}$ of DEET in the presence of treatment to ratio of $Q_{30}$ of DEET in the absence of any treatment.
[c] Ratio of $J$ of DEET in the presence of penetration modifier formulation in a solvent to $J$ of DEET in the presence of solvent alone.
[d] Ratio of $Q_{30}$ of DEET in the presence of penetration modifier formulation in a solvent to $Q_{30}$ of DEET in the presence of a solvent alone.

**Table 7**
Permeation parameters of DEET in the presence of DMMCBI in selected vehicles.

| Parameters formulation ($n=5$) | Permeability coefficient, $K_p \times 10^{-5} \pm$ SD (cm/h) | Mean flux, $J \pm$ SD (μg/cm$^2$/h) | Cumulative amount of DEET after 30 h, $Q_{30} \pm$ SD (μg/cm$^2$) | [a]$MR_J$ | [b]$MR_{30}$ | [c]$MR^*_J$ | [d]$MR^*_{Q_{30}}$ |
|---|---|---|---|---|---|---|---|
| No treatment, C | 6.0 ± 2.0 | 58.0 ± 19.4 | 1413.9 ± 315.6 | 1.0 | 1.0 | – | – |
| Water, W | 17.3 ± 2.36 | 168.2 ± 22.9 | 4243.4 ± 364.2 | 2.9 | 3.0 | 1.0 | 1.0 |
| Propylene glycol, PG | 26.4 ± 6.4 | 256.0 ± 61.7 | 5894.6 ± 1458.6 | 4.4 | 4.2 | 1.0 | 1.0 |
| Ethanol, E | 11.6 ± 2.4 | 112.6 ± 23.2 | 2040.5 ± 1266.3 | 1.9 | 1.4 | 1.0 | 1.0 |
| PEG 400, PEG | 2.6 ± 1.2 | 25.6 ± 12.2 | 701.7 ± 312.0 | 0.4 | 0.5 | 1.0 | 1.0 |
| DMMCBI in water, DCW | 14.8 ± 4.6 | 143.1 ± 44.7 | 3718.3 ± 1093.3 | 2.5 | 2.6 | 0.8 | 0.9 |
| DMMCBI in PG, DCPG | 7.0 ± 2.7 | 67.7 ± 25.9 | 2042.0 ± 824.2 | 1.2 | 1.4 | 0.3 | 0.3 |
| DMMCBI in ethanol, DCE | 3.8 ± 0.02 | 37.3 ± 0.2 | 1100.4 ± 51.3 | 0.6 | 0.6 | 0.3 | 0.5 |
| DMMCBI in PEG 400, DCPEG | 3.7 ± 0.3 | 36.0 ± 2.5 | 993.3 ± 93.5 | 0.6 | 0.8 | 1.4 | 1.4 |




SD represents standard deviation.
[a] Ratio of $J$ of DEET in the presence of treatment to $J$ of DEET in the absence of treatment.
[b] Ratio of $Q_{30}$ of DEET in the presence of treatment to ratio of $Q_{30}$ of DEET in the absence of any treatment.
[c] Ratio of $J$ of DEET in the presence of penetration modifier formulation in a solvent to $J$ of DEET in the presence of solvent alone.
[d] Ratio of $Q_{30}$ of DEET in the presence of penetration modifier formulation in a solvent to $Q_{30}$ of DEET in the presence of a solvent alone.

with N-0915–PEG 400 and PEG 400 treatments, no difference was observed ($p > 0.05$) and the retardation was mainly due to PEG 400 in the formulation.

### 3.3.3. Effect of DMBIS in selected vehicles on permeation of DEET

The effects of DMBIS and vehicles on DEET permeation were investigated and data are provided in Table 6. DEET permeation in the presence of the formulations was compared and it was observed its permeation was significantly enhanced in the presence of DMBIS–water, DMBIS–PG, DMBIS–ethanol and DMBIS–PEG 400 (as compared to controls (no treatment, $p < 0.05$)). No retardation was observed in the presence of any DMBIS formulation. However, differential enhancement of DEET was observed in DMBIS formulations (Table 6) with MR ratio ranging from 3.4 in DMBIS–PG to 1.5 in case of DMBIS–ethanol. With DMBIS–water treatment, enhancement of DEET permeation was similar to water alone ($p > 0.05$). The enhancement seemed to be mainly due to the presence of water in the formulation. Unlike DMBIS–water, in DMBIS–PG treatment, permeability coefficient, flux and $Q_{30}$ values of DEET were significantly enhanced as compared to PG alone. This suggests that DMBIS and PG present in DMBIS–PG act synergistically to enhance the permeation of DEET. This suggestion is further supported by the fact that besides PG, DMBIS itself has been reported to be a good enhancer. The enhancement of DEET in the presence of DMBIS–ethanol was probably due to ethanol because the permeability coefficient, flux and $Q_{30}$ values after DMBIS–ethanol and ethanol treatment were similar ($p > 0.05$). The permeation of DEET in the presence of DMBIS–PEG was markedly different ($p < 0.05$) from that of PEG 400 suggesting the importance of the enhancer DMBIS in improving the permeation of DEET.

### 3.3.4. Effect of DMMCBI in selected vehicles on permeation of DEET

Table 7 provides the permeation parameters for the DMMCBI formulations. Comparing DEET permeation in the absence of any treatment, DMMCBI–water ($p < 0.05$) and DMMCBI–PG ($p > 0.05$) enhanced the permeation of DEET and DMMCBI–ethanol ($p > 0.05$) and DMMCBI–PEG 400 ($p > 0.05$) retarded the permeation of DEET. The enhancement of DEET in DMMCBI–water treatment was due to the presence of water in the formulation because no statistical difference in permeability coefficient, flux and $Q_{30}$ values was observed between DMMCBI–water and water alone ($p > 0.05$).

However, in DMMCBI–PG treatment, there was a significant decrease in permeability coefficient, flux and $Q_{30}$ values of DEET as compared to PG alone ($p < 0.05$). The antagonistic nature of the two components in DMMCBI–PG (with DMMCBI being a retardant and PG being an enhancer) led to weakening of the retarding action of DMMCBI. This weakening led to statistically insignificant ($p > 0.05$) enhancement of DEET permeation as compared to control (no treatment). In DMMCBI–ethanol, a significant decrease in DEET permeation was observed compared to ethanol alone ($p < 0.05$). The retardation observed in DMMCBI–ethanol was due to a similar phenomenon as described in N-0915–ethanol. Comparing the permeation of DEET in the presence of DMMCBI–PEG 400 and PEG 400 no statistical difference was obtained ($p > 0.05$). Use of DMMCBI–PEG 400 resulted in a slight retardation of DEET permeation in comparison to control (no treatment). Also, slight enhancement of DEET permeation was observed when DMMCBI–PEG 400 was compared to PEG 400 alone. Statistical comparison of permeability coefficient, flux and $Q_{30}$ values of DEET in the presence of DMMCBI–PEG 400, PEG 400 alone and controls showed all three treatments to be similar ($p > 0.05$) suggesting no action (enhancement/retardation) by DMMCBI–PEG 400.

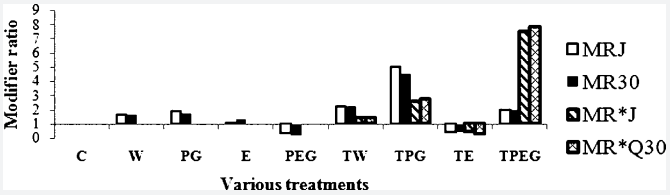
### 3.3.5. Effect of TBDOC in selected vehicles on permeation of DEET

It was observed that formulations TBDOC–water, TBDOC–PG and TBDOC–PEG 400 enhanced the permeation of DEET and TBDOC–ethanol retarded permeation (Table 8). The permeability coefficient, flux and $Q_{30}$ values of DEET in the presence of all TBDOC formulations were statistically different from permeability coefficient, flux and $Q_{30}$ values in the absence of any treatment ($p < 0.05$). The various transdermal parameters in the presence of TBDOC are summarized in Table 8.

Comparing the permeability coefficient, flux and $Q_{30}$ values of DEET in the presence of TBDOC–water with water alone reveals no statistical difference ($p > 0.05$), suggesting enhancement was due to the presence of water. Moreover, no detectable amounts of TBDOC were observed in the TBDOC–water mixture. In TBDOC–PG treatment, permeability coefficient flux and $Q_{30}$ values of DEET were significantly greater than those in the absence of any treatment

**Table 8**
Permeation parameters of DEET in the presence of TBDOC in selected vehicles.

| Parameters formulation ($n = 5$) | Permeability coefficient, $K_p \times 10^{-5} \pm$ SD (cm/h) | Mean flux, $J \pm$ SD (μg/cm$^2$/h) | Cumulative amount of DEET after 30 h, $Q_{30} \pm$ SD (μg/cm$^2$) | [a]$MR_J$ | [b]$MR_{30}$ | [c]$MR^*_J$ | [d]$MR^*_{Q30}$ |
|---|---|---|---|---|---|---|---|
| No treatment, C | 10.1 ± 2.9 | 97.8 ± 27.8 | 2736.9 ± 695.0 | 1.0 | 1.0 | – | – |
| Water, W | 15.9 ± 1.9 | 154.5 ± 18.7 | 4513.1 ± 671.4 | 1.6 | 1.6 | 1.0 | 1.0 |
| Propylene glycol, PG | 18.9 ± 3.4 | 183.7 ± 33.4 | 4590.7 ± 728.5 | 1.9 | 1.7 | 1.0 | 1.0 |
| Ethanol, E | 11.3 ± 4.6 | 109.8 ± 44.9 | 3667.5 ± 1120.9 | 1.1 | 1.3 | 1.0 | 1.0 |
| PEG 400, PEG | 2.6 ± 1.2 | 25.6 ± 12.2 | 701.7 ± 312.0 | 0.3 | 0.2 | 1.0 | 1.0 |
| TBDOC in water, TW | 22.4 ± 0.5 | 216.9 ± 5.1 | 6151.6 ± 215.9 | 2.2 | 2.2 | 1.4 | 1.4 |
| TBDOC in PG, TPG | 50.3 ± 4.3 | 487.6 ± 41.6 | 12423.6 ± 1163.4 | 5.0 | 4.5 | 2.6 | 2.7 |
| TBDOC in ethanol, TE | 6.3 ± 0.8 | 44.91 ± 8.4 | 192.0 ± 7.2 | 0.4 | 0.4 | 0.4 | 0.3 |
| TBDOC in PEG 400, TPEG | 19.8 ± 0.7 | 192.0 ± 7.2 | 5520.3 ± 240.5 | 2.0 | 2.0 | 7.5 | 7.8 |




SD represents standard deviation.
[a] Ratio of $J$ of DEET in the presence of treatment to $J$ of DEET in the absence of treatment.
[b] Ratio of $Q_{30}$ of DEET in the presence of treatment to ratio of $Q_{30}$ of DEET in the absence of any treatment.
[c] Ratio of $J$ of DEET in the presence of penetration modifier formulation in a solvent to $J$ of DEET in the presence of solvent alone.
[d] Ratio of $Q_{30}$ of DEET in the presence of penetration modifier formulation in a solvent to $Q_{30}$ of DEET in the presence of a solvent alone.

($p < 0.05$). This suggests that enhancement of DEET in the presence of TBDOC–PG, occurs due to contribution of both components of the formulation. Unlike other TBDOC formulations, retardation of DEET was observed in TBDOC–ethanol treatment. The statistical analysis showed significant decrease in permeability coefficient, flux and $Q_{30}$ values as compared to ethanol alone ($p < 0.05$). TBDOC has been reported to show significant retardation of several actives such as paraoxon, DEET, 2-hydroxy-4-methoxybenzophenone and others by Purdon (Purdon, 2005). The reason for retardation of DEET in the presence of TBDOC–ethanol as opposed to enhancement by TBDOC–PG will be investigated using thermal and spectral analytical techniques. TBDOC–PEG 400 treatment showed significant enhancement of DEET compared to PEG 400 alone ($p < 0.05$) suggesting the contribution of both components towards enhancement. The exact mechanisms are currently being explored.

## 4. Conclusion

The results in this study were based on determination of mean flux values, permeability coefficient and cumulative amount of permeant absorbed after 30 h. In this study, percutaneous permeation parameters determined were solely based on in vitro experimentation as compared to the use of mathematical models. There are reports of models of differential complexity that contain several relationships linking the permeant flux across the human skin to the physico-chemical properties of the compound being evaluated (Potts and Guy, 1992, 1995; Abraham et al., 1997; Lien and Gao, 1995). These models have the advantage of conveniently and cost-effectively predicting the extent of percutaneous absorption of the molecule without actually performing the in vitro and in vivo measurements. All the known models relate the permeability coefficient to properties such as octanol–water partition coefficient, melting point, molecular weight or aqueous solubility that can readily be obtained or calculated from group-contribution approach or Hansch fragment values for partition coefficient (Leo et al., 1971). The equations derived in the mathematical models were best fit of experimentally determined flux values the above-mentioned physico-chemical properties.

In spite of sophistication of these models, the results are mere estimates and they still require verification by in vitro and eventually by in vivo experimentation. Therefore in our study, no mathematical model was utilized for predicting permeability parameters.

The results in this study indicate that the effect of a penetration modifier on the permeation of an active can change from vehicle to vehicle irrespective of its potency as an enhancer/retardant, or its potential to form one-sided hydrogen bonds or two-sided hydrogen bonds with lipids present in the stratum corneum. All these results suggest that formulations of penetration modifiers in various vehicles create a series of unique interactions on the surface of stratum corneum that lead to the enhancement/retardation of the permeant. In order to explain the type of interaction occurring in the stratum corneum after such applications, studies using differential scanning microscopy and infra-red spectroscopy are being performed in our laboratory. Our results also showed that the amount of enhancement/retardation was not solely dependent on the concentration of penetration modifier present in the formulation. This was evident in the laurocapram–PEG 400 formulation, where despite sufficient solubility of laurocapram in the vehicle, no enhancement was observed.

Therefore, our results suggest that there is a need for modification/extension for the pre-existing theory of H-bond formation with ceramide molecules of the stratum corneum to explain the phenomenon of enhancement/retardation, because our study indicates that enhancers can act as retardants or vice versa with change of the formulation vehicle. Moreover, enhancer/retardants should be collectively termed as penetration modifiers since their activity changes with the formulation.

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