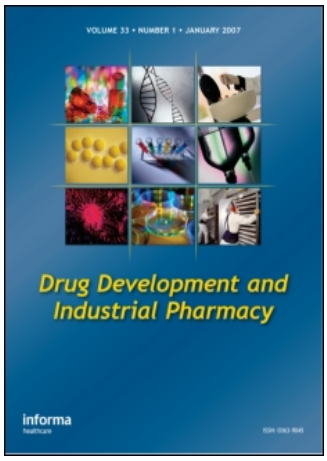

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Transdermal and Buccal Delivery of Methylxanthines Through Human Tissue In Vitro

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ABSTRACT We examined the in vitro permeation of central nervous stimulants–caffeine, theophylline, and theobromine across human skin with the aid of six chemical enhancers. It was found that oleic acid was the most potent enhancer for all three methylxanthines. Further optimization studies with different solvents showed that caffeine transport could be enhanced to give flux values up to 585 $\mu g/cm^2.hr^{-1}$. Theobromine and theophylline delivery rates proved insufficient. An additional study involving a buccal tissue equivalent showed that this membrane was more permeable than skin for all model actives tested and would offer an alternate way of delivery.

KEYWORDS Transdermal, Buccal, Oleic acid, Terpenes, Caffeine, Theobromine, Theophylline

INTRODUCTION

Caffeine, theobromine, and theophylline are all compounds that belong to the methylxanthine class of central nervous system (CNS) stimulants (Mandel, 2002). These drugs have been shown to delay fatigue, extend physical strength and endurance, as well as heighten alertness and cognitive abilities in humans. Indeed, these compounds form key constituents of many traditional beverages and refreshments such as coffee, tea, mate, guarana, cocoa, chocolates as well as derived nutritional products. It should be stated that caffeine is actually metabolized in humans to form theophylline and theobromine and that these metabolites are weaker CNS stimulants than the parent drug.

It has been proposed that it would be useful to design methylxanthine-loaded transdermal patches that could effectively deliver these agents into the systemic circulation. A potential use for such patches is for soldiers in the field preparing for combat, where a controllable and sustained boost in physical stamina and mental alertness would be highly advantageous. The current study was designed as a preliminary investigation aimed at assessing the feasibility of this type of application.

Unfortunately, the methylxanthines are not favorable candidates for transdermal delivery due to their relatively hydrophilic nature. Caffeine, theophylline, and theobromine exhibit log octanol-water partition coefficient values of

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–0.07, –0.02 and –0.78, respectively (Hansch et al., 2002). As a general rule, the lipoidal nature of the stratum corneum means it is difficult for compounds with log octanol-water partition coefficient values below 1 to penetrate through the skin in pharmacologically effective concentrations (Flynn & Stewart, 2004; Meidan & Michniak, 2004). Nevertheless, efficacious transdermal delivery may be possible if sufficiently potent chemical enhancer-vehicle combinations could be identified. To this end, we studied the effect of various enhancers and vehicle compositions on the in vitro permeation of caffeine, theophylline, and theobromine across human skin. Six different enhancers were selected, including Azone® and oleic acid, representing two relatively potent and commonly-employed enhancers (Moser et al., 2001). The other four accelerants were terpenes. These naturally-occurring volatile oils exhibit low cutaneous irritancy and have been given the designation of generally recognized as safe (GRAS) by the FDA (El-Kattan et al., 2001). Four chemically-distinct terpenes were selected: cineole (an ether), terpineol (an alcohol), menthone (a ketone), and limonene (a hydrocarbon). Most of the studies involved the use of propylene glycol as the vehicle but in some additional studies, we screened mixtures containing propylene glycol:ethanol, ethanol:water, or propylene glycol:water. In addition to the skin transport studies, we also measured methylxanthine solubility in each enhancer-containing vehicle.

In a separate experiment, the alternative approach of delivering methylxanthines through the buccal route was investigated. A cultured buccal tissue equivalent was used for this set of experiments.

MATERIALS AND METHODS

Chemicals

Caffeine, propylene glycol (PG), ethanol (EtOH), oleic acid (OA), limonene, menthone, cineole, and terpineol were purchased from Sigma-Aldrich (St. Louis, MO). Theobromine and theophylline were kindly supplied by Clemson University (Clemson, SC). Azone® was obtained as a gift from the New Jersey Center for Biomaterials (Piscataway, NJ). Phosphate buffered saline (PBS) tablets were purchased from MP Biomedicals, CA. All the other employed chemicals and solvents were of analytical grade.

Biological Membranes

The National Disease Research Interchange (Philadelphia, PA) provided samples of dermatomed (~500 μm) human skin, derived from male Caucasian cadavers aged between 50 and 70 years. These skins 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 hr in PBS by mounting on jacketed Franz cells which were maintained at 37°C. EpiOral™, representing a highly differentiated, three-dimensional, cultured buccal tissue equivalent was obtained from MatTek Corp (Ashland, MA). EpiOral™ consists of normal, human-derived epithelial cells that have been cultured to form multilayered, highly differentiated models of the human buccal phenotypes (http://www.mattek.com). Morphologically, the tissue models closely parallel human tissues, thus providing a useful in vitro model. These were stored under refrigeration (4°C) for not more than 24 hr prior to use.

Preparation of Formulations

A saturated solution of each methylxanthine was prepared by dissolving, at 32 ± 0.5°C, an excess of the drug in propylene glycol over a 48 hr period. During the final 10 hr, the solution was thoroughly shaken every hour using a vortex mixer. At 48 hr, the saturated drug solution was isolated as the supernatant. Six distinct enhancers (menthone, terpineol, cineole, limonene, oleic acid, and Azone®) were added separately to the saturated methylxanthine solution to form a 5% v/v solution. Control solutions consisted of each drug dissolved in propylene glycol without enhancer.

In addition to the general protocol described above, two sets of supplementary studies were also performed on caffeine. The first such study involved investigation of vehicle composition on caffeine skin permeation. To this end, caffeine solutions containing 5% v/v oleic acid were prepared in the following vehicles: 33% propylene glycol and 67% ethanol; 50% propylene glycol and 50% water; 70% ethanol and 30% water. Caffeine dissolved to saturation in pure propylene glycol formed a control solution. In the second supplementary study, the influence of oleic acid concentration on caffeine transport was evaluated. The oleic acid concentrations tested were 2, 5, and 10% v/v in ethanol:water (70:30).

For the buccal tissue studies, each methylxanthine was prepared as a saturated solution in propylene glycol containing 5% v/v oleic acid.

Methylxanthine Solubility Measurements

The solubility of each tested methylxanthine in propylene glycol was measured, both in the presence and absence of enhancers. Additionally, for caffeine, solubility determinations were also performed in different vehicles containing 5% v/v oleic acid. Briefly, an excess of methylxanthine was added to known volumes of vehicle, vortexed for 2 min to dissolve the drug and then equilibrated at 32 ± 0.5°C for more than 48 hr. In the case of chemical enhancers, 5% v/v enhancer solutions in the respective vehicles were prepared and centrifuged prior to filtration through a 0.45 μm filter (Millex®-LCR, Millipore, MA). The formulations were vortexed every 1 hr for the last 10 hr. Subsequently, the contents were centrifuged in a Model TJ-6 centrifuge (Beckman Instruments Inc., Palo Alto, CA) at 10,000 rpm for 15 min. The supernatant was removed, filtered, and diluted 10-fold in the respective vehicle. The methylxanthine content in each sample was then quantified by HPLC.

Transport Studies

The barrier membranes, either dermatomed human skin or Epioral™ samples, were mounted in vertical Franz diffusion cells (PermeGear Inc., Bethlehem, PA). These exhibited a diffusion-available surface area of 0.64 cm^2 and a receptor compartment volume of 5.1 mL. The receptor compartments were filled with isotonic phosphate buffered saline (0.155M and pH 7.4), which was stirred at 600 rpm. The fluid in each receptor compartment was maintained at 37 ± 0.5°C by the use of a thermostatic water pump (Haake DC10, Karlsruhe, Germany) that circulated water through the jacket surrounding each main chamber. The biological membranes were initially left in the Franz cells for 1 hr in order to facilitate their hydration before the experiment. After this period, a 100 μL aliquot of saturated methylxanthine solution with or without enhancer was deposited in each donor compartment. The donor compartment was covered with Parafilm® to prevent evaporation. Receptor solution samples of 300 μL were then collected at hourly intervals and these were stored at –20°C prior to HPLC analysis. For the transdermal studies, sampling was conducted between 4 and 24 hr inclusive. For the buccal membrane studies, hourly sampling was undertaken during the first 5 hr of the transport studies. In all cases, an equivalent amount of fresh PBS was placed in the receptor solution after each sample withdrawal. Each permeation experiment was conducted as 4 or 5 replicate runs.

Quantification of Methylxanthines

Collected samples were injected into a HPLC (Hewlett Packard 1100) system equipped with degasser (G1379A), autosampler (G1313A), quaternary pump (G1311A), and a UV-visible diode array (G1315A) and a Microsorb® RP column (Merck, Darmstadt, Germany).

For caffeine, the mobile phase consisted of ethanol:water:acetonitrile at a percentage composition of 20:70:10. The elution parameters were a flow rate of 1mL/min and an injection volume of 20 μL. The detection wavelength was 270 nm and the retention time was 3.4 min. The limit of detection for caffeine was 0.5 μg/mL.

For theobromine, the mobile phase consisted of acetonitrile:water at a percentage composition of 5:95. The elution parameters were a flow rate of 0.8 mL/min and an injection volume of 5 μL. A detection wavelength of 254 nm was used while the retention time was determined to be 9.8 min. The limit of detection for theobromine was 5 μg/mL.

For theophylline, the mobile phase had a percentage composition of acetonitrile: water (5:95). A flow rate of 0.8 mL/min was employed while the injection volume was 5 μL. The detection wavelength was 254 nm and the retention time was 15.8 min. The limit of detection for theophylline was 5 μg/mL. An external standard was employed for all the test compounds. All methods were validated and correlation coefficients of 0.9990, 0.9991, and 0.9999 for linearity of plot were observed for caffeine, theobromine, and theophylline, respectively. Intraday and interday variability were also calculated and determined to be 0.95, 2.68, 4.1% and 1.37, 1.8, 2.86%, for caffeine, theobromine, and theophylline, respectively.

In all cases, drug concentration values were corrected for the progressive dilution that occurred during the sampling and replenishing undertaken during

the course of the experiment. Subsequently, the flux and the cumulative amount of drug transported were plotted as a function of time. From these graphs, the steady-state drug flux was calculated from the slope of the linear portion of each plot. Cumulative drug amounts were also recorded. All results were expressed as mean values ± standard deviations. Statistical analysis involved application of student t-tests and one-way ANOVA. These tests were performed using "Analysis Tool Pak" software from Microsoft® Office.

RESULTS

Transdermal Delivery of Caffeine

Table 1 shows the solubilities of caffeine in the various tested vehicle-enhancer combinations. It can be seen that when the vehicle was pure propylene glycol with no enhancer present, maximal caffeine solubility was 8.4 mg/mL. Interestingly, incorporation of 5% v/v enhancer into this vehicle augmented caffeine solubility to between 15.23 and 18.88 mg/mL depending upon which individual enhancer was selected. Fig. 1 presents the caffeine–human skin permeation profiles using various enhancers present at a 5% v/v concentration in pure propylene glycol. The data shows that relative to conditions of enhancer absence, all the enhancers except limonene significantly accelerated caffeine flux through the skin ($p < 0.05$). Terpineol, menthone and cineole produced moderate increases in caffeine permeation relative to conditions of enhancer absence with enhancement ratios of 3.2, 3.5, and 9.5, respectively (See Table 1). However, there were no statistically significant differences in terms of flux enhancement between each of these three terpenes ($p > 0.05$). The presence of Azone® in the vehicle enhanced caffeine flux by 37.3 times while oleic acid enhanced caffeine flux by 55 times. Thus, the rank order of flux enhancement was: oleic acid > Azone® > menthone, cineole, terpineol > limonene.

TABLE 1 **Solubilities and Flux Values of Test Compounds in Various Vehicle-Enhancer Combinations. All Enhancers Present at 5% v/v Concentration (n = 4 or 5)**

Vehicle	5% v/v Enhancer	Solubility in mg/mL n = 3	Flux μg/cm^2.hr n = 4–5	Enhancement ratio (ER)
Caffeine				
PG:EtOH(33:67)	Oleic acid	13.82 ± 0.75	75 ± 14	102
EtOH:Water(70:30)	Oleic acid	28.19 ± 0.09	162.7 ± 38.7	222
PG:Water(50:50)	Oleic acid	19.43 ± 0.06	47.6 ± 6.2	65.2
PG	None	8.39 ± 0.4	0.73 ± 0.1	Control
	Oleic acid	15.23 ± 0.6	40.25 ± 5.06	55.1
	Azone®	15.68 ± 0.0	27.25 ± 6.2	37.2
	Cineole	17.28 ± 1.06	6.95 ± 4.75	9.5
	Limonene	17.62 ± 0.62	0	0
	Menthone	17.23 ± 0.44	2.53 ± 1.35	3.5
	Terpineol	18.88 ± 1.02	2.38 ± 0.97	3.2
Theobromine				
Propylene glycol	None	0.52 ± 0.06	0	0
	Oleic acid	0.46 ± 0.06	5.06 ± 0.4	–
	Azone®	1.02 ± 0.09	0	0
	Cineole	0.84 ± 0.04	0	0
	Limonene	1.13 ± 0.13	0	0
	Menthone	1.04 ± 0.07	0 ± 0	0
	Terpineol	0.93 ± 0.04	0	0
Theophylline				
Propylene glycol	None	0.30 ± 0.15	11.9 ± 0.02	Control
	Oleic acid	0.8 ± 0.0	39.6 ± 2.2	3.3
	Azone®	0.76 ± 0.0	0	0
	Cineole	0.95 ± 0.01	0	0
	Limonene	0.91 ± 0.01	0.003 ± 0.001	–
	Menthone	0.95 ± 0.02	23.1 ± 3.8	1.9
	Terpineol	0.92 ± 0.02	0	0

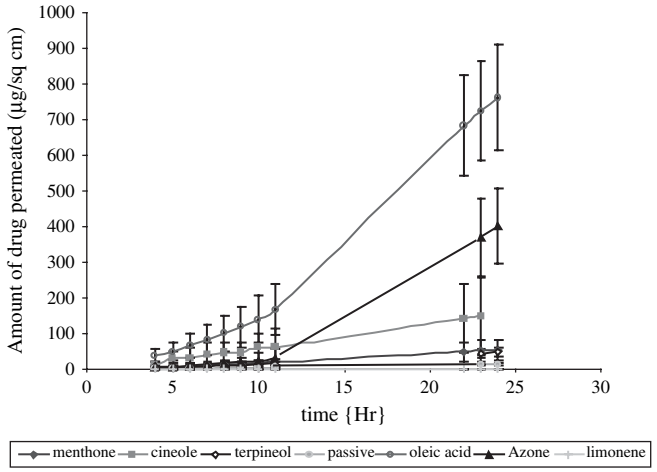

FIGURE 1 **The Influence of Enhancers on Caffeine Penetration Through Human Skin In Vitro. Each Formulation Consisted of Caffeine Saturated in Propylene Glycol Containing 5% v/v Enhancer *n* = 4–5.**

Vehicle optimization studies were performed for caffeine and the derived solubility and permeation data are presented in Table 1 and Fig. 2. The formulation composed of 5% v/v oleic acid in ethanol:water (70:30) yielded a high initial flux of 162.7 ± 38.7 µg/cm^2.hr^{-1} although a pronounced plateau effect developed from 7 hr onwards. This plateau however is not due to loss of sink conditions since at 7 hr the caffeine concentration ratio for donor solution and skin:receptor solution was still 198:1. Use of the vehicle consisting of 5% v/v oleic acid in ethanol:propylene glycol (33:67) yielded a lower steady-state caffeine flux of 75 ± 14 µg/cm^2.hr. Application of 5% v/v oleic acid in water:propylene glycol (50:50) resulted in the lowest permeation rate of 47.6 ± 6.2 µg/cm^2.hr^{-1}.

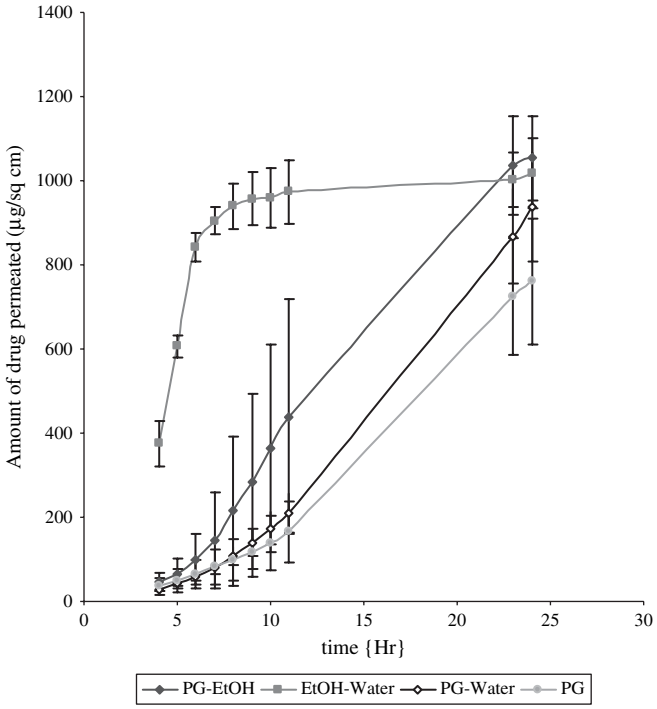

FIGURE 2 **The Influence of Vehicles on Caffeine Penetration Through Human Skin In Vitro. Each Formulation Consisted of Caffeine Saturated in Either: Ethanol:Water (70:30); Ethanol:Propylene Glycol (33:67); or Water:Propylene Glycol (50:50). Each Vehicle Contained 5% v/v Oleic Acid *n* = 4–5.**

Investigations into the effect of changing oleic acid concentrations on caffeine transport were also conducted. The most efficacious vehicle identified in the previous section was selected for these experiments i.e., ethanol:water (70:30). Table 2 summarizes the results obtained. It can be seen that caffeine flux rises with increasing concentrations of oleic acid with the 10% v/v enhancer concentration producing a mean steady-state methylxanthine flux of 585 ± 44 µg/cm^2.hr^{-1}.

Transdermal Delivery of Theobromine

Studies to determine the saturation solubilities of theobromine in various propylene glycol formulations were performed and the resulting data are listed in presented in Table 1. Without any added enhancer, the theobromine concentration in the vehicle was 0.52 mg/mL. It is noteworthy that when the propylene glycol contained 5% v/v oleic acid, theobromine solubility was marginally reduced to 0.46 mg/mL. In contrast, the presence of any other of the tested enhancers solubilized the drug, yielding saturation solubilities in the 0.8–1.13 mL/mL range. Subsequent transport studies involving these saturated solutions were then performed and the flux values and permeation profiles are shown in Table 1 and Fig. 3, respectively. It was determined that only the formulations containing oleic acid yielded measurable transdermal drug penetration with

TABLE 2 **Steady-State Caffeine Flux Through Human Skin as a Function of Oleic Acid Concentration. The Vehicle Composition was Ethanol:Water (70:30)**

Concentration of oleic acid v/v	Flux µg/cm^2.hr *n* = 4–5
2	50.5 ± 4.6
5	162.7 ± 38.7
10	585 ± 44

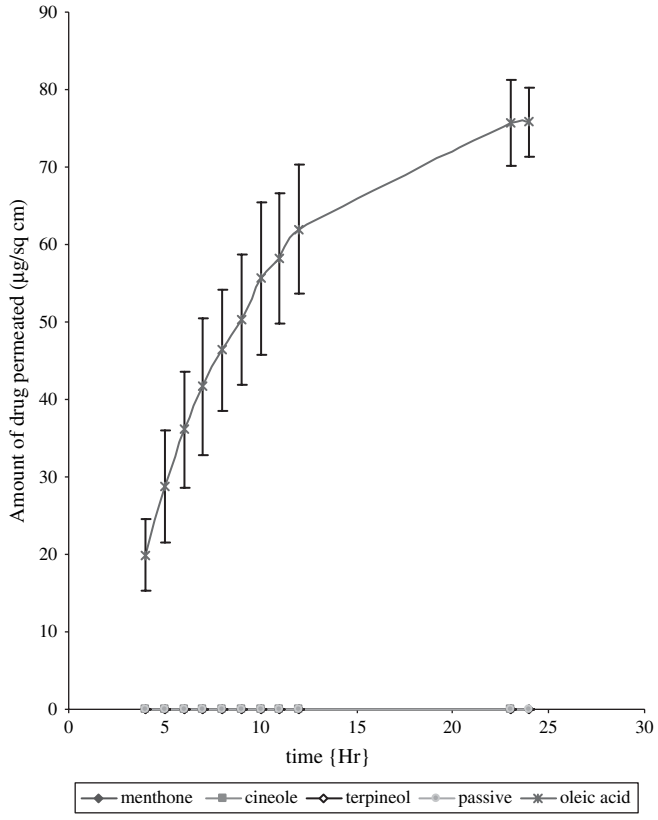

FIGURE 3 **The Influence of Enhancers on Theobromine Penetration Through Human Skin In Vitro. Each Formulation Consisted of Theobromine Saturated in Propylene Glycol Containing 5% v/v Enhancer *n* = 4–5.**

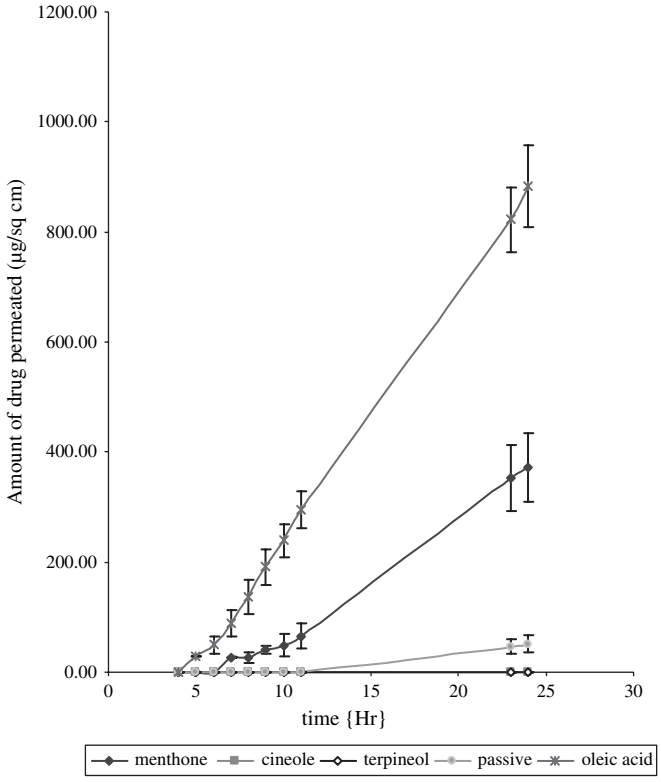

FIGURE 4 **The Influence of Enhancers on Theophylline Penetration Through Human Skin In Vitro. Each Formulation Consisted of Theophylline Saturated in Propylene Glycol Containing 5% v/v Enhancer *n* = 4–5.**

a mean recorded flux of 5.06 µg/cm^2.hr^{-1}. No drug flux was detected using the other formulations.

Transdermal Delivery of Theophylline

Table 1 presents the theophylline solubility data for all of the investigated formulations. It can be seen that the drug's saturation solubility in pure propylene glycol was 0.30 mg/mL. However, inclusion of Azone®, oleic acid or the four terpenes increased this figure to 0.76, 0.80, and 0.91–0.95 mg/mL respectively. The results of theophylline penetration studies are presented as both figures in Table 1 as well as profiles in Fig. 4. With no incorporated enhancer, mean steady-state drug flux was 11.9 µg/cm^2.hr^{-1}. Intriguingly, inclusion of cineole, terpineol, limonene, or Azone® actually suppressed virtually all methylxanthine permeation. In contrast, the presence of 5% v/v concentrations of menthone or oleic acid modestly enhanced theophylline flux, yielding measured enhancement ratios of 1.9 and 3.3, respectively. Hence, the rank order of transport enhancement was: oleic acid > menthone > limonene, terpineol, cineole, Azone®.

Buccal Delivery of Caffeine, Theobromine and Theophylline

Fig. 5 presents the results obtained from the buccal tissue transport studies. Data analysis indicated that the steady-state permeation of caffeine, theophylline and theobromine was 409.7 ± 14.8, 64.19 ± 2.62, and 34.8 ± 4.6 µg/cm^2.hr^{-1}, respectively. Not surprisingly, the flux of all three drugs through the unkeratinized buccal tissue was much higher than through human skin and this observation is depicted graphically in Fig. 6.

DISCUSSION

Our results showed that oleic acid was the most potent enhancer for all three methylxanthines, consistently outperforming both Azone® and the selected terpenes. The effectiveness of oleic acid as an accelerant is well-documented (Gabiga et al., 2000; Lee et al.,

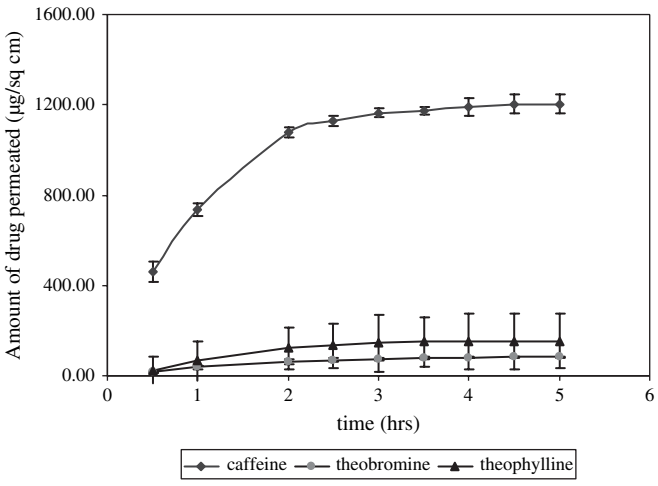

FIGURE 5 **Penetration of Methylxanthines through EpiOral™. Each Formulation Consisted of Drug Saturated in Propylene Glycol and 5% v/v Oleic Acid (*n* = 4).**

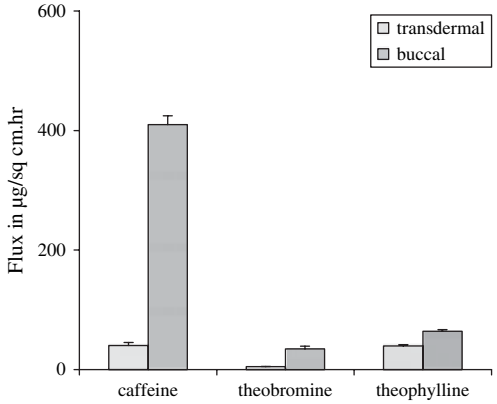

FIGURE 6 **A Comparison of Steady-State Flux of Methylxanthines Through Buccal and Skin Tissue (*n* = 4–5).**

2006; Lee et al., 2005; Narishetty & Panchagnula, 2004; Tanojo et al., 1997). Investigators have reported that incorporation of oleic acid yielded a 950-fold increase in drug permeation through hairless mouse skin (Oh et al., 2001). Oleic acid is thought to fluidize the stratum corneum lipid bilayers, thus perturbing the barrier (Jiang & Zhou, 2003). FT-IR observations have indicated that this process seems to be a particular feature of cis-unsaturated fatty acids such as oleic acid (Babu et al., 2006). The enhancer may also reduce the proportion of crystalline lipids and create more permeable oleic acid-rich domains (Rowat et al., 2006). Furthermore, oleic acid and propylene glycol are known to act synergistically (Aungst, 1995; Larrucea et al., 2001). Propylene glycol is a rapid skin permeant and long chain fatty acids may further accelerate its cutaneous penetration. This phenomenon probably explains why oleic acid and propylene glycol produced such high methylxanthine flux values despite the comparable drug solubilities of the terpene-containing and Azone®-containing formulations.

It is interesting to compare oleic acid and terpenes as enhancers. For all the drugs, oleic acid seemed to be the optimal enhancer despite the fact that the drugs exhibited similar solubilities in the enhancer: propylene glycol combinations for all enhancers (solubilities range from 15.6–18.8 mg/mL for caffeine and 0.8–0.95 mg/mL for theophylline). The high flux produced by oleic acid for all drugs is probably due to two factors: First, oleic acid might be a better enhancer than the terpenes due to its multiple mechanisms of action. Secondly, the combination of oleic acid and propylene glycol is synergistic while terpenes with propylene glycol may not offer this effect.

The enhancement in caffeine delivery from different solvents may be explainable by the varying caffeine solubilities in the binary solvent combinations. Various solvents containing 5% oleic acid were tested and it was found that the highest flux was provided by ethanol:water:oleic acid in the initial hours followed by propylene glycol:oleic acid combinations ($p < 0.05$). Though any combination containing propylene glycol with oleic acid should have been synergistic and would be expected to yield the highest permeation, the solubility of caffeine in the ethanol:water:oleic acid combination (28.2 mg/mL) was the highest followed by propylene glycol:water:oleic acid (19.4 mg/mL), propylene glycol:oleic acid (15.23 mg/mL) and ethanol: propylene glycol:oleic acid (13.8 mg/mL). A lower solubility leads to a lower amount of drug in the receptor and hence a lower maximum concentration gradient of the drug across the skin and therefore, a lower drug flux across the skin. However in this case solubility does not seem to be the factor affecting the flux. Propylene glycol:water:oleic acid inspite of higher solubilisation of caffeine than ethanol:propylene glycol:oleic acid shows a lower flux than the latter. This implies the increased flux in both ethanol containing formulations is due to the well-known capability of ethanol itself to act as a permeation enhancer. However, statistically there was no significant difference in the permeation curves of propylene glycol:water:oleic acid and ethanol:propylene glycol:oleic acid ($p > 0.05$). Thus, ethanol: water with oleic acid as enhancer seemed to be the optimal combination. This is in agreement with previous

reports that showed that an ethanol:water:oleic acid combination accelerated drug delivery across both rat and human cadaver skin (Kim & Chien, 1995).

From further optimization studies with caffeine, we found that a high delivery rate of 585 ± 44 μg/cm^2.hr^{-1} could be achieved by employing a 10% v/v oleic acid concentration in an ethanol:water (70:30) vehicle. This figure compares very favorably with other in vitro caffeine transdermal flux values recently documented in the literature. Mean steady-state flux values of 6.54 μg/cm^2.hr^{-1} (Dias et al., 1999), 7.78 μg/cm^2.hr^{-1} (Batchelder et al., 2004) and 8.21 μg/cm^2.hr^{-1} (Akomeah et al., 2004) have been reported for studies involving human epidermal, full-thickness porcine, and human epidermal sections, respectively. Recent studies have been able to achieve a delivery rate of 85 μg/cm^2 after 6 hr while commercial formulations have been seen to deliver 6.6 μg/cm^2 in the same time frame (Nicoli et al., 2004; Nicoli et al., 2005). A 10 cm^2 skin patch with the formulation parameters used in our study could systemically deliver a much higher amount in our case (5850 μg of caffeine per hour). Since, caffeine metabolism is relatively minor over this duration, such an application would result in an approximate caffeine plasma concentration of 1160 μg/L in a typical adult. This approaches the 1500μg/L caffeine plasma concentration in adults following the drinking of a typical single cup of coffee (Benowitz, 1995).

With regards to theophylline, the highest mean steady-state flux attained was 39.6 μg/cm^2.hr^{-1} and this figure exceeds some of the values reported in other human skin transport studies (Vavrova et al., 2005). A 10 cm^2 transdermal patch designed to transfer theophylline at this rate would systemically deliver 396 μg of drug in 1 hr. Assuming negligible metabolism and clearance, this application could yield a theophylline plasma concentration of 79.2 μg/L in an average adult. Unfortunately, this concentration is still well under an order of magnitude too low to produce significant CNS stimulation (Tiplady et al., 1990).

With respects to theobromine, it was possible to obtain a maximal transdermal flux rate of just over 5 μg/cm^2. hr^{-1}. Although, data from the literature correlating theobromine plasma concentrations with its CNS effects seems to be lacking, it is known that theobromine is a milder stimulant than caffeine. Hence, even a 10 cm^2 skin patch delivering the drug at 50 μg/hr would not be likely to prove efficacious.

Finally, for caffeine formulations, buccal application allowed at least 10-times more drug permeation than the transdermal route. The absence of a keratinized epithelium barrier makes the buccal mucosa an attractive alternative delivery route to the skin (Nicolazzo et al., 2005). Thus, this study is in agreement with previous in vitro and in vivo reports that have documented high buccal drug permeation rates (Langoth et al., 2005; Lee & Kellaway, 2000; Nyberg, 1986).

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