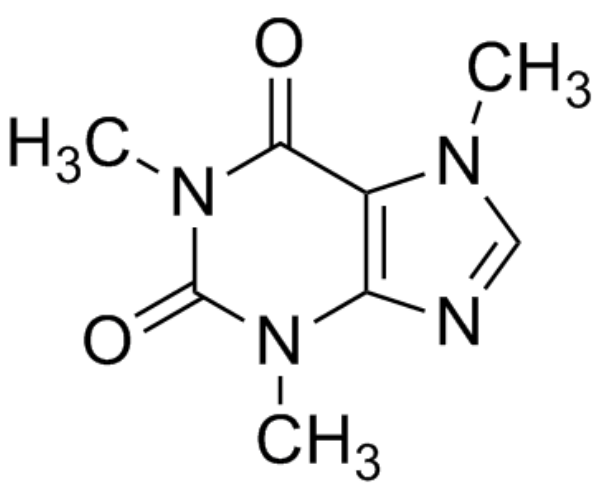
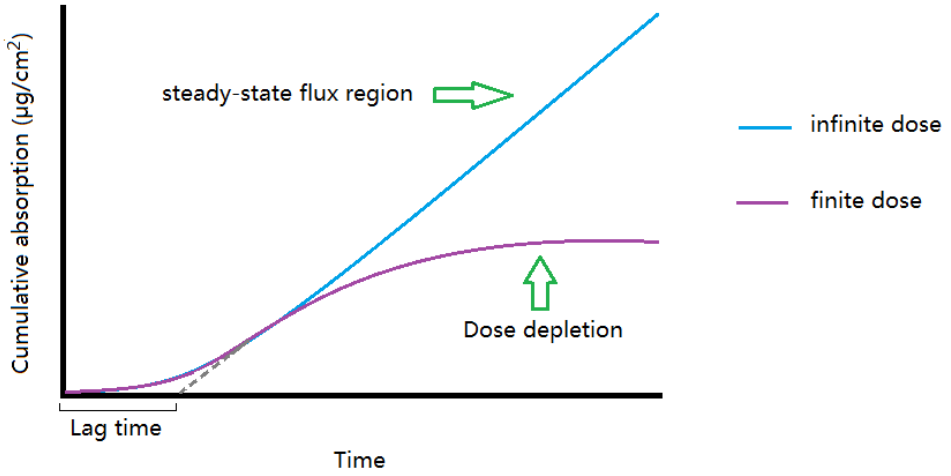
*Graphical Abstract (for review)





List of Figures

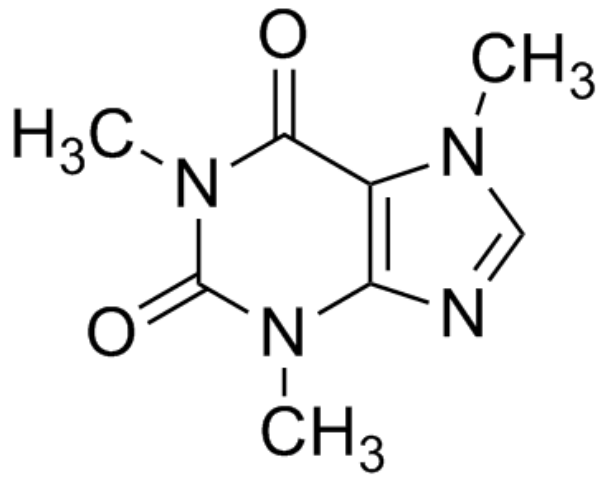


Figure 1: Caffeine

Table(s)

Table 6: Summary of *in vitro* permeation skin studies conducted with caffeine formulations

Formulation	Tissue	Author(s)	Caffeine concentration / amount applied	Permeation parameter reported
Ointment bases	Human	Zesch et al. (1979)	0.1 %, 4.3 μg /cm^2	SC penetration: 0.63 – 2.18 μg/cm^2
Petrolatum, ethylene glycol gels, aqueous gels	Human	Bronaugh and Franz (1986)	0.5 – 60 μg /cm^2	k_p : 2.1 – 7.2 x 10^{-4} cm/h
Ethoxylated amines	Human	Hadgraft et al. (1986)	0.2 %	k_p: 2.5 – 4.9 x 10^{-3} cm/h
OA, LA	Human	Green et al. (1988)	0.2 %, 1.1 μg /cm^2	k_p: 3.0– 4.5 x 10^{-3} cm/h
Water, PGDP, Labrafil M 1944, Labrasol®, PG, Transcutol™, PG/PGDP	Human	Bonina et al. (1993)	0.2 – 2.8%, 0.3 – 3.7 mg/cm^2	J: 0.3 – 2.3 μg/cm^2/h
PG, PG/water, PG/ethanol, PG/OA, PG/water/OA, ethanol/water/OA, PG/Azone™, PG/menthone, PG/limonene, PG/cineole	Human	Thakur et al. (2007)	0.8– 2.8%, 1.1 – 3.7 mg/cm^2	J: 0 – 585 ± 44 μg/cm^2/h
Water, PG/ethanol/water, 1,2-pentanediol/Water, PG/water, ethanol/water	Porcine	Duracher et al. (2009)	2 %, 0.2 mg/cm^2	k_p: 1.7 – 7.7 x 10^{-4} cm/h Cumulative permeation: 32.4 – 179.7 μg/cm^2
Cationic “gemini” surfactants/PG	Porcine	Silva et al. (2013)	1.5 %, 7 mg/cm^2	J: 6.1 – 10.9 μg/cm^2/h Cumulative permeation: 127.9 – 226.9 μg/cm^2
Spilanthol/ethanol/water	Human	De Spiegeleer et al. (2013)	5.4%, 42 mg/cm^2	Cumulative permeation: ~300 – 1000 μg/cm^2
Phytoceramides/ethanol/water	Human	Veryser eta l. (2015)	5.4%, 42 mg/cm^2	k_p: 2.1 – 7.0 x 10^{-4} cm/h J: 10..9 – 35.4 μg/cm^2/h
Emulsions (O/W)	Human	Bonina et al. (1992)	0.1 %, 133 μg/cm^2	k_p: 1.5 – 2.9 x 10^{-3} cm/h J: 0.2 -1.6 μg/cm^2/h
Emulsions (O/W, W/O/W)	Human	Doucet et al. (1998)	1 %, 2.6 mg/cm^2	k_p: 2.4 – 6.0 x 10^{-4} cm/h J: 2.4 – 6 μg/cm^2/h
Emulsions (O/W, W/O), hydrogel	Human	Dreher et al. (2002)	1 %, 0.1 mg/cm^2	Percentage permeation: 15 – 20%
Emulsion (O/W), microemulsion,hydrogel	Porcine	Bolzinger et al. (2007)	0.8 %, 3.2 mg/cm^2	k_p: 2.0 – 3.6 x 10^{-3} cm/h J: 15.6 – 28.8 μg/cm^2/h

Emulsion (W/O), Pickering emulsion (W/O)	Porcine	Frelichowska et al. (2009)	0.8 %, 3 mg/cm^2	k_p: 1.0 – 3.0 x 10^{-3} cm/h J: 10– 25 μg/cm^2/h
Microemulsions (O/W, W/O, bicontinuous)	Porcine	Naoui et al. (2011)	0.8 %,3 mg/cm^2	k_p: 4.0 – 12.0 x 10^{-3} cm/h J: 35 – 99 μg/cm^2/h
Microemulsions (O/W, W/O, bicontinuous)	Porcine	Zhang and Michniak-Kohn (2011)	1 %	J: 5.1 – 11.6 μg/cm^2/h Cumulative permeation: 91.8 – 237.8 μg/cm^2
Microemulsions (O/W, O/W gel)	Porcine	Sintov and Greenberg (2014)	1 %, 2.8 mg/cm^2	k_p: 1.3 – 1.5 x 10^{-3} cm/h Cumulative permeation: 264.5 – 322.7 μg/cm^2
Nanoparticles (Starch derivatives)	Human	Santander-Ortega et al. (2010)	-	Cumulative permeation: <0.2 μg/cm^2
Nanoparticles (Silica composites)	Porcine	Pilloni et al. (2013)	3 %, 4.7 – 9.4 mg/cm^2	k_p: 2.0 – 2.2 x 1^{0-4} cm/h J: 6.0 – 6.9 μg/cm^2/h

Topical and transdermal delivery of caffeine

Lin Luo and Majella E. Lane

Department of Pharmaceutics

UCL School of Pharmacy

29-39 Brunswick Square

London

WC1N 1AX

*Corresponding author

Tel: +44 207 7535821

Fax: +44 870 1659275

Email: majella.lane@btinternet.com

Abstract

Caffeine is administered topically and transdermally for a variety of pharmaceutical and cosmetic applications and it is also used as a model hydrophilic compound in dermal risk assessment studies. This review considers the physicochemical and permeation properties of caffeine with reference to its delivery to and through the skin. Since it has been used as a model compound the findings have implications for the delivery of many hydrophilic compounds having similar properties. Various passive and active formulation strategies to promote enhanced skin permeation of caffeine are considered. Models to study percutaneous caffeine penetration are also discussed in detail.

Key words: Caffeine, topical, transdermal, formulation, permeation

1. Introduction

Caffeine (1,3,7-trimethylpurine-2,6-dione) is a methyl xanthine alkaloid which is consumed as a beverage, administered as a medicine or applied for cosmetic purposes (Figure 1). Caffeine is also employed as a model hydrophilic compound in skin toxicology; dermal absorption of such "marker" compounds is used for risk assessment of exposure to hazardous substances in man (OECD, 2004). Caffeine is recommended as a test substance by the OECD because it has been studied extensively *in vitro* and *in vivo*. Although coffee and other caffeine containing drinks, such as tea, have been consumed at least since the fifteenth century the molecule itself was not isolated until 1820 (Anft, 1955). This article focusses on caffeine interactions with the skin with an emphasis on topical delivery strategies; systemic delivery of caffeine is also possible in certain cases. The major route for actives to permeate through the skin is via the lipid content of the outermost layer (Menon et al., 2012), the stratum corneum (SC). However, caffeine does not possess the properties of an ideal skin penetrant as it is a hydrophilic material with a Log P of -0.07 (Table 1). Caffeine also exhibits unusual solubility behaviour in non-aqueous solvents (Bustamante et al., 2002) and forms aggregates in aqueous solutions (Guttmann and Higuchi, 1957; Cesaro et al., 1976). The various formulation approaches which have been employed to date to enhance skin penetration of caffeine will be reviewed. Only studies on human and porcine skin are considered as other models are not reliable predictors of skin penetration in man.

Table 1. Physicochemical properties of caffeine

Molecular Weight*	194.2
Log P*	-0.07
Solubility*	1 in 46 of water, 1 in 1.5 of boiling water
Solubility parameter**	31 $MPa^{½}$
Melting point*	238°C
pK_a *	14 (25°C), 10.4 (40°C)

*Moffat et al. 2004

**Bustamante et al., 2002

2. Pharmacology, pharmacokinetics and metabolism

Caffeine inhibits the phosphodiesterase enzyme and has an antagonistic effect on central adenosine receptors. It is a stimulant of the Central Nervous System and may produce wakefulness and heightened mental activity. The molecule also increases rate and depth of respiration but it is a weaker bronchodilator than theophylline (Parfitt and Martindale, 1999). Caffeine has been

investigated for its neuroprotective effects in dementia (Ritchie et al., 2007) as well as its potential as an aid to recognise hypoglycaemic symptoms in diabetic patients (Debrah et al., 1996; Watson et al., 2000). The major advantages claimed for use of caffeine in topical cosmetic products are that it (i) prevents excessive fat accumulation in skin, (ii) promotes lymphatic drainage and (iii) protects skin from photodamage. Scientific evidence for many of these proposed benefits is lacking and most studies in the literature are based on cell culture or mouse models (Herman and Herman, 2013). The efficacy of caffeine in the management of gynoid lipodystrophy (cellulite) via proposed adipocyte lipolysis and increased cyclic adenosine monophosphate (cAMP) is controversial. The applications of caffeine in the management of this condition have been reviewed by Herman and Herman (2013). The authors describe in detail potential roles for caffeine in lipolysis including effects on catecholamine secretion, cAMP levels, lipase activity, PDE inhibition and lymphatic drainage. Although caffeine has been shown *in vitro* to promote follicular proliferation evidence for this effect *in vivo* has not been reported(Fischer et al., 2007).

When administered orally, the bioavailability of caffeine is ~100% with values for plasma half-life, volume of distribution and clearance reported respectively as 2 -10 h, 0.5 L/kg and 1 – 2 mL/min/kg (Moffat et al., 2004). The extent to which the molecule binds to plasma proteins is ~35% (Blanchard, 1982). For treatment of neonatal apnoea of prematurity the recommended dose is 5 – 20 mg/kg caffeine (as the citrate) administered orally or intravenously. The recommended oral dose of caffeine for mild stimulant purposes is 50 – 200 mg daily (Parfitt and Martindale, 1999). Following oral administration, caffeine may be subject to metabolism by N-demethylation, acetylation and oxidation; approximately 1% of the molecule is excreted unchanged. There is no evidence, to date, that caffeine undergoes appreciable metabolism following application to skin. Poisonings and fatalities have followed ingestion of large oral doses of caffeine but no toxic or skin reactions have been reported following dermal exposure in healthy patients (Moffat et al., 2004).

3. Skin permeation of caffeine

This section reviews percutaneous penetration of caffeine from simple aqueous solutions and solvents. The influence of application site, age, occlusion, tissue preparation for *in vitro* studies transport in hair follicles and lateral diffusion properties on caffeine skin penetration is also examined. Strategies to promote caffeine permeation based on chemical or physical enhancement of skin delivery or other means are discussed in Sections 4 and 5.

3.1 Influence of anatomic site

Feldmann and Maibach (1970) investigated the permeation of caffeine in human subjects. A dose of 4 μg/cm^2 of CAF was applied in acetone to the ventral forearm over a total area of 13 cm^2. Absorption values of ~50% were reported following urinary collection over 5 days. Franz (1975) investigated caffeine permeation *in vitro* and reported median percentage absorption of 9% of caffeine from a finite dose application. In a later report by Franz (1978) the values for caffeine absorption *in vitro* and *in vivo*, respectively were reported as 24.1 ± 7.8% and 22.1 ± 15.8%. The differences in the earlier values for *in vivo* caffeine absorption were attributed to the correction used for urinary excretion and the duration of sample collection. Rougier and co-workers (1987) investigated the skin permeation of caffeine as a function of other anatomic sites in human subjects. A solution of caffeine in ethylene glycol, Triton X-100 and water was applied to the arm, abdomen, post-auricular region or forehead. After 30 min contact the sites were washed with ethanol/water followed by a rinse with water and then dried. Total amounts of caffeine penetrated were ranked as follows: forehead>arm>post auricular region>abdomen. This is consistent with recent data reported by Machado et al. (2010); these workers calculated effective pathlengths for water diffusion as a function of anatomic site.

3.2 Influence of age

The influence of aging on skin permeation of caffeine was examined by Roskos et al. (1989). A finite dose (4 μg/2.5 cm^2) of caffeine in acetone was applied to the forearms of young (22-40 years) and old (65-86 years) subjects. A protective patch (non-occlusive) was then applied over the application site for 24 h followed by washing of the skin and collection of urinary samples. The dose recovered was 32.1 ± 4.2% for the young group while the corresponding value for the old group was 61.8 ± 5.4%. This was a relatively small study with 5 volunteers for the young group and 7 for the older group and data were expressed as mean values with standard error of the mean. As sebaceous gland activity and lipid content of skin changes with age (Pochi et al., 1979) the authors suggested that this might explain the diminished amounts of caffeine absorption in the older subjects. However, changes in corneocyte size and skin hydration with age are also likely to influence caffeine permeation with age. The barrier function of skin is not fully developed in new-born infants particularly if they are immature. Amato et al. (1991, 1992) reported transdermal delivery of caffeine in preterm infants for the management of apnoea. Caffeine (as the citrate salt) was applied twice daily in a gel formulation; total daily doses were 20 mg for babies with birth weights < 1000 g and 15 mg for babies with birth weights > 1000 g. Therapeutic levels were considered to be 25 – 100 μmol/L and were achieved within 48 h of the first application of the gel. Transdermal delivery of caffeine is

attractive in this case as oral absorption of drugs in low birth weight infants is erratic, unpredictable and sometimes not possible because of gastrointestinal disease (Evans and Rutter, 1986).

3.3 Influence of occlusion

Treffel et al. (1992) investigated the effects of occlusion on the *in vitro* skin permeation of caffeine. The dose of caffeine applied was 2.6 μg/cm^2 in an acetone solution (160 μg/mL). Specialised diffusion cells with a completely closed donor compartment were used for the occlusion study and conventional Franz cells were used for the corresponding unoccluded study. Cumulative amounts of caffeine permeated after 24 h were of the order of 1 μg/cm^2 and were not significantly different for occlusion compared with no occlusion.

3.4 *In vitro* studies with human skin and porcine tissue

A multi-laboratory study investigated caffeine permeation *in vitro* under finite dose conditions (van de Sandt et al., 2004). Caffeine was applied as a 0.4 %w/v solution in ethanol:water (1:1) and dosed at 25 μl/cm^2 to human skin samples. Maximum flux values and percentage permeated for caffeine were 2.24 ± 1.43 μg/cm^2/h and 24.5 ± 11.6% respectively. Cumulative amounts of caffeine which had permeated by the end of the experiment were not reported.

Schreiber et al. (2005) reported the *in vitro* permeation of caffeine in human and porcine skin. Infinite doses of caffeine were applied (285 μl/cm^2 of a 1% w/v solution in phosphate buffered saline) to the donor compartments of Franz cells and permeation experiments were conducted up to 6 h. Cumulative amounts of caffeine permeated were ~150 μg/cm^2 for dermatomed, cryopreserved, porcine abdominal skin and ~10 μg/cm^2 for heat separated human epidermis. No differences in caffeine permeation were observed for full thickness human skin compared with heat separated human skin. Unfortunately, the authors did not conduct the corresponding finite dose study. Skin penetration of caffeine *in vitro* using heat separated or dermatomed human skin was investigated by Atrux-Tallau et al. (2007). The dose of caffeine applied was 128 μg/cm^2 as an aqueous solution. Values for cumulative amounts of caffeine permeated were ~30 μg/cm^2 (~23% of the applied dose) for heat-separated skin which were not significantly different for dermatomed tissue. This is to be expected as the hydrophilic nature of caffeine would result in faster permeation through dermal tissue.

3.5 Caffeine transport through hair follicles

Otberg et al. (2008) investigated the transport of caffeine through hair follicles. A solution of caffeine in ethanol and propylene glycol (PG) was applied to the chest of male volunteers; caffeine

was dosed at 10 μg/cm^2 over 25 cm^2. Blood samples were taken up to 72 h following application of the formulation. Following a 7-day interval hair follicles on the same sites were blocked using a varnish/wax mixture. The caffeine formulation was then applied and blood samples collected as for the first study. Serum caffeine values for open follicles were reported as 3.75 ng/mL, 5 min after application of the formulation, but caffeine could not be detected following application to the closed follicle sites until 20 min after application. Caffeine values were generally lower for the sites with closed follicles compared with the site with unblocked follicles. In a later study Trauer et al. (2009) investigated the effects of blocking hair follicles on caffeine permeation *in vitro* using Franz cells. Excised female breast skin was used and the same formulation used in the earlier *in vivo* study was applied but the dose of caffeine was five-fold higher because of limitations with the analytical method used. Permeation studies were conducted for 24 h with caffeine detection in the receptor compartment after 2 h. Significantly higher amounts of caffeine ($p<0.05$) were observed for skin with unblocked follicles (0.39 %) compared with the closed follicular skin (0.09 %). At 24 h the amount of caffeine recovered from the control skin was ~17% and for the skin with blocked follicles the recovery was ~7%. These data point to an important role for the follicular pathway in caffeine skin permeation but further studies are required to confirm this.

3.6 Influence of skin washing

Nielsen (2010) investigated the effects of skin washing on caffeine permeation *in vitro*. Solutions of caffeine (4 mg/mL) were applied at a dose of 50 μL/cm^2 to human skin mounted in diffusion cells. At 6 h the skin surface and donor compartment were gently cleaned using soap of neutral pH and a cotton swab followed by two rinses with isotonic water. Following drying, permeation continued and samples were taken for all cells up to 42 h. At the end of the experiment the amounts of caffeine permeated were 24 % for the control group and 8 % for the cells which had been washed. This would suggest that a significant amount of caffeine resided on the skin and was washed from the surface.

3.7 Lateral skin diffusion

Gee et al. (2012) used a novel tape stripping technique to investigate the lateral diffusion of caffeine both *in vitro* and *in vivo*. Dermatomed human abdominal skin was employed for *in vitro* studies with 1.8 μl of caffeine (0.8 % w/v) being applied to the donor compartment of Franz cells. Permeation studies were conducted up to 6 h. For *in vivo* studies a solution of caffeine in ethanol (1.8 μl, 0.8 % w/v) was applied to an area of 0.5 cm^2 on the volar forearm of 8 volunteers (4 males, 4 females). After 3 min, 3 h and 6 h, tape perforated into concentric circles was used to strip the area

of application as well as adjacent areas. A total of 10 tape strips was taken for each site from which drug and protein content were determined. Lateral diffusion and limited skin penetration was observed for caffeine at 3 min compared with other drugs studied (ibuprofen and hydrocortisone). The authors suggested that the comparatively wider lateral diffusion observed for caffeine might reflect the presence of a moisture film at the skin surface which would aid dissolution and spreading of the molecule.

4. Formulation strategies for delivery of caffeine to the skin

4.1 Ointments and gels

One of the earliest investigations to compare the influence of vehicle on caffeine delivery was reported by Zesch et al. (1979). Four ointment bases containing 0.1% w/w caffeine were evaluated namely Vaseline™, aqueous wool wax alcohol ointment (water-in-oil emulsion), aqueous hydrophilic ointment (oil-in-water emulsion) and a polyethylene glycol ointment. For *in vitro* studies 30 mg of ointment was applied to human skin over a 7 cm^2 area. One preparation (aqueous wool wax alcohol ointment) was evaluated further *in vivo* with 100 mg of ointment being applied to a 28 cm^2 site. Following application ointments were rubbed in for 90 s to ensure uniform distribution and the time allowed for penetration was 10, 30, 100, 300 and 1000 min. At the end of the permeation period skin biopsies and tape stripping were conducted for both sets of experiments. The best penetration (2.18 $\mu g/cm^2$) into the SC *in vitro* was observed for the aqueous woolwax alcohol ointment with the poorest uptake observed for the polyethylene glycol ointment (0.63 $\mu g/cm^2$). Similar permeation into the SC *in vitro* was observed for Vaseline™ and the aqueous hydrophilic ointment despite the differences in their composition. However for the epidermis *in vitro* data at 100 min indicated that the aqueous hydrophilic ointment (oil-in-water emulsion) delivered the greatest amount of caffeine with the poorest delivery from the aqueous woolwax alcohol ointment. Much lower caffeine values for SC and epidermis (0.31 and 0.05 $\mu g/cm^2$ respectively) were observed *in vivo* compared with the *in vitro* results which were attributed to clearance of caffeine by the skin vasculature.

Bronaugh and Franz (1986) evaluated caffeine permeation from petrolatum, gels containing ethylene glycol and simple aqueous gels in human skin, *in vitro* and *in vivo*. Doses applied *in vitro* were 25 mg/cm^2 or greater with caffeine amounts varying from 0.5 – 60 $\mu g/cm^2$ depending on the vehicle studied. Maximum permeation was observed for the petrolatum vehicles followed by the ethylene glycol gels with lowest permeation from the aqueous gel. This trend was consistent with the degree of saturation of caffeine in the respective vehicles. Results for *in vivo* absorption were in general agreement with the *in vitro* studies.

4.2 Penetration enhancers

Penetration enhancers are chemicals which are proposed to modify transiently the solubility of an active in the skin and/or promote enhanced diffusion of the active (Lane, 2013). Hadgraft et al. (1986) investigated the effects of an ethoxylated amine on caffeine penetration *in vitro*. Pre-treatment of human skin with either 10 or 100 mM of bis-(2-hydroxyethyl)oleylamine in ethanol increased the permeability coefficient of caffeine by a factor of 40 compared with ethanol alone. However these studies were conducted under infinite dose conditions therefore permeation enhancement is likely over-estimated compared with the amounts of caffeine that are typically applied *in vivo*. Green et al. (1988) assessed the effects of oleic acid (OA) and lauric acid (LA) on the skin penetration of caffeine. Human skin mounted in Franz cells was pre-treated with a solution of the fatty acid in ethanol for a period of 2 h. Permeation of caffeine was increased for both fatty acids but as for the previous studies experiments were conducted under infinite dose conditions. The authors also noted that caffeine did not form ion pairs with the fatty acids.

Suspensions of caffeine in a range of solvent enhancers have been evaluated by Bonina et al. (1993). Permeations experiments were conducted with human skin over 24 h; flux values reported are shown in Table 2.

Table 2. Solubility and skin flux of caffeine from a range of vehicles (Adapted from Bonina et al., 1993)

Vehicle	**Solubility*** **(mg/mL)**	**Flux** **(μg cm^{-2} h^{-1})**
Water	27.9 ± 1.7	0.72 ± 0.04
Propylene glycol dipelargonate	1.9 ± 0.1	2.28 ± 0.35
Labrafil M 1944	3.3± 0.4	1.16 ± 0.09
Labrasol	12.0 ± 0.8	0.28 ± 0.06
Propylene glycol	13.9 ± 1.2	0.70 ± 0.1
Transcutol	14.1 ± 0.9	0.64 ± 0.2
Propylene glycol / Propylene glycol dipelargonate	13.9 ± 0.9	2.19 ± 0.17

*Measured at 30°C for 48 – 72 h

Compared with the flux values for the aqueous suspension, no enhancement of caffeine permeation was observed for Labrasol®, Transcutol® or PG. Improved transport was observed for

propylene glycol dipelargonate (PGDP), Labrafil® M 1944 and a mixture of PGDP and PG. Labrasol® is a mixture of ethoxylated C_8 and C_{10} glycerides, glycerides and free polyethylene glycol (PEG), Transcutol® is a monomethyl ether and Labrafil® M 1944 consists of ethoxylated glycerides and glycerides. The superior effects of the PG / PGDP vehicle were attributed to the ability of PGDP to interact with intercellular skin lipids combined with the ability of PG to promote uptake of PGDP into the skin.

Four terpenes as well as oleic acid and Azone® were evaluated by Thakur et al (2007) for their ability to enhance delivery of caffeine to the skin. Enhancers were incorporated (5 % v/v) in saturated solutions of caffeine in PG; the control consisted of a saturated solution of caffeine in PG. OA was also evaluated in three other vehicles, namely PG:ethanol (33:67), PG:water (50:50) and water:ethanol (30:70). Formulations (100 μL) were applied to dermatomed human skin samples mounted in Franz cells with an effective diffusion area of 0.64 cm^2. Permeation experiments were conducted under occlusion for 24 h and results are summarised in Table 3. Maximal permeation was observed for the hydro-alcoholic vehicle containing OA consistent with synergistic effects on permeation. However, since infinite dose conditions were employed the data have limited applicability for finite dose conditions.

Table 3. Solubility and flux values of caffeine for a range of vehicles (Adapted from Thakur et al., 2007)

Vehicle	**Enhancer**	**Solubility (mg/mL)**	**Flux (μg/cm²/h)**
PG	-	8.4 ±0.4	0.7 ±0.1
PG	OA 5%	15.2 ±0.6	40.3 ±5.1
PG:Ethanol	OA 5%	13.8± 0.8	75.0 ±14.0
PG:Water	OA 5%	19.4± 0.1	47.6 ±6.2
Water:Ethanol	OA 2%	-	50.5 ±4.6
Water:Ethanol	OA 5%	28.2 ±0.1	162.7 ±38.7
Water:Ethanol	OA 10%	-	585 ± 44
PG	Azone 5%	15.7 ±0.0	27.3 ±6.2
PG	Cineole 5%	17.3 ±1.1	7.0 ±4.8
PG	Limonene 5%	17.6 ±0.6	0
PG	Menthone 5%	17.2 ±0.4	2.5 ±1.4
PG	Terpineol 5%	18.9 ±1.0	2.4 ±1.0

Duracher and co-workers (2009) investigated 1,2-pentanediol, ethanol and PG as penetration enhancers for caffeine using full thickness porcine skin. Formulations containing 2% caffeine were prepared as outlined in Table 4. For permeation studies 10 μL of each vehicle was applied to porcine ear skin with a surface area of 0.95 cm^2. Experiments were conducted for 24 h with occlusion of the donor compartment. Mass balance studies were also conducted by washing the surface of the skin, tape stripping to remove the SC as well as extraction of caffeine from the epidermis and dermis. The incorporation of small amounts of 1,2-pentanediol clearly promoted caffeine penetration compared with the aqueous vehicle. Approximately 32% and 65% of caffeine penetrated the skin for the vehicles containing respectively 2.5% and 5% of 1,2-pentanediol however the highest cumulative permeation was observed for a ternary ethanol:PG:water system. This probably reflects the synergistic effects of the enhancers in this formulation. A ternary system containing 1,2-pentanediol and ethanol would have been a useful comparator study and would allow investigation of any potential synergy betwen these enhancers.

Table 4. Caffeine skin permeation for vehicles studied by Duracher et al. (2009)

Vehicle	**Cumulative permeation at 24 h (μg)**
Water	39.8 ±16.7
PG:Ethanol:Water (25:25:48)	170.7 ±38.3
1,2-pentanediol:Water (2.5:95.5)	68.7 ±17.1
1,2-pentanediol:Water (5:93)	153.5 ±31.4
PG:Water (5:93)	93.0 ±18.9
Ethanol:Water (5:93)	30.8 ±3.7

*Determined at 25 ±1°C over 24 h

A series of cationic 'gemini' surfactants were evaluated by Silva et al. (2013) for their effects on caffeine penetration. These molecules differ from conventional surfactants as they contain two hydrophilic tails and at least two hydrophobic tails linked covalently by a spacer group. The surfactants studied were dimethylene-1,2- bis(dodecyldimethylammonium bromide), hexamethylene-1,2-bis(dodecyldimethylammonium bromide) and decamethylene-1,2-bis(dodecyldimethylammonium bromide). Solutions of surfactants were prepared in PG at a concentration of 0.16 M with the single chain surfactant dodecyltrimethylammonium bromide, Azone® and PG alone used for comparison. Dermatomed porcine tissue mounted in Franz cells was pre-treated with the various preparations for 1 h prior to application of 0.3 mL of a caffeine gel (1.5

% w/w) to the donor compartments (diffusional area 0.64 cm^2). After 24 h two of the gemini surfactants as well as the single chain surfactant demonstrated a two-fold enhancement of caffeine permeation (8.2 – 10.9 $\mu g/cm^2/h$ compared with no pre-treatment (4.5 $\mu g/cm^2/h$). As the studies were conducted under infinite dose conditions finite dose experiments may elucidate more clearly if these surfactants have real potential for topical delivery. A further issue is that such molecules typically have much lower critical micelle concentrations than conventional surfactants which may have implications for thermodynamic activity of the active.

Spilanthol, an N-alkylamide obtained from the *Acmella* plant species has recently been evaluated for its skin penetration enhancement properties with reference to caffeine (De Spiegeleer et al., 2013). A range of concentrations of the enhancer were incorporated in ethanol:water (50:50) solutions with caffeine (4.3 % w/v). Permeation experiments were conducted with dermatomed human skin in Franz cells and formulations were dosed at 500 μl over 0.64 cm^2. The maximum cumulative caffeine permeation (~650 μg) was observed for solutions containing spilanthol 1% w/v which was significantly greater than that observed for the control solution (~150 μg). For all concentrations of the enhancer studied most of the caffeine was deposited on the surface of the skin or in the epidermis. The very high permeation values reported are consistent with the large doses of caffeine applied (35 mg/cm^2).

A new class of lipids containing hydrophobic oleyl chains and heterocyclic head groups was synthesised by Singh et al. (2013). The skin penetration enhancement properties with respect to caffeine were examined *in vitro* using human skin. All lipid formulations also contained Transcutol®. Although the authors claimed that permeation enhancement was observed for each of the lipids the influence of Transcutol® was not considered. Information on the control formulations lacked sufficient detail and no permeation profiles were provided for any of the formulations.

Most recently Veryser et al (2015) have evaluated phytoceramides as potential skin penetration enhancers of caffeine. Ceramides consist of a sphingosine molecule linked to a fatty acid via an amide linkage; phytoceramides contain no unsaturated C_4-C_5 linkage and contain an additional hydroxyl group at C_4. Formulations were prepared as reported in an earlier study (De Spiegeleer et al., 2013) with caffeine incorporated at 80% of its saturation solubility in ethanol:water (50:50) vehicles. Phytoceramides were incorporated at a concentration of 1% w/v and Azone® was used as a positive control. All formulations were evaluated in Franz cell studies with human skin; the dose applied was 500 μl/0.64 cm^2. After 24 h, cumulative permeation for a solution of caffeine alone was ~150 μg and maximum values for the phytoceramide formulations did not exceed ~400 μg. Statistical analysis was not conducted to determine the significance of the permeation differences. In contrast the corresponding permeation value for Azone® was 1,609 μg. Percentages of caffeine

permeated were 5.98 ± 97 % for the Azone® formulation and did not exceed 1.6 % for the phytoceramide preparations.

4.3 Emulsions, microemulsions, Pickering emulsions

The effects of emulsion vehicles on caffeine skin penetration were investigated by Bonina and co-workers (1992). Formulations were prepared using an emulsion base consisting of glycerine, PEG-24 glyceryl stearate, glyceryl stearate, cetostearyl alcohol, octyl stearate, methyl isothiazolin-one, methyl-chloro-isothiazolin-one and imidazolidinylurea and water. Caffeine was incorporated at 0.001 % or 0.1 % in the emulsions and 100 mg of formulation was applied to human skin in Franz cells with an effective area of 0.75 cm^2. Perfluoropolymethylisopropylether (1, 3 or 5 % w/w) was also incorporated in a number of formulations. Steady state flux values of caffeine did not exceed ~1.6 $\mu g/cm^2/h$ for the 0.1% caffeine preparations and were ~0.2 $\mu g/cm^2/$ h for the 0.01 % caffeine emulsions. The inclusion of the perfluorinated ether had no influence on caffeine penetration.

Delivery of caffeine from an emulsion compared with an acetone solution was examined by Chambin-Remoussenard et al. (1993). The O/W emulsion consisted of water, polyoxyethylene glycol stearate, stearyl alcohol, petroleum and ethanol. Vehicles were applied to the volar forearm of healthy volunteers with average doses being 2.22 ± 0.14 and 2.37 ±0.10 $\mu g/cm^2$ for the acetone solution and emulsion respectively. Recovery was measured for up to 6 h post treatment by washing treated sites and swabbing; following the washing procedure the area was tape stripped to determine the amounts of caffeine deposited in the skin. Significant differences in amounts recovered by washing and swabbing were evident for the emulsion (1443 ± 251 ng/cm^2) compared with the acetone solution (995 ± 172 ng/cm^2). Results for the tape stripping procedure also confirmed a five-fold difference in amount of caffeine deposited in skin following application of the emulsion (212 ng/cm^2) versus acetone (37 ng/cm^2). Significant lateral spread of caffeine in the SC away from the treatment site was observed for the acetone solution (~40%) but not for the emulsion. The results are probably affected by the low surface tension of acetone which will spread over the skin whilst it evaporates.

Delivery of caffeine from an O/W and a water-in-oil-in water (W/O/W) emulsion prepared with similar components and containing caffeine 1% (w/w) was reported by Doucet et al. (1998). The primary emulsion consisted of Vaseline™ oil, cetearyl octanoate, magnesium sulphate, distilled water and caffeine with cetyl dimethicone copolyol as the lipophilic emulsifier. For the multiple (W/O/W/) emulsion the O/W preparation was further mixed with an external aqueous phase consisting of an ethylene oxide/propylene oxide block copolymer and water. Formulations were applied to human skin mounted in Franz cells at a dose of 260 11 mg/cm^2 and permeation was

measured over 24 h. Cumulative percentages absorbed were 3.21 ± 0.18% for the O/W emulsion and 1.25 ± 0.17% for the W/O/W emulsions. Actual flux values were ~6 μg/cm^2/h for the primary emulsion and 2.4 μg/cm^2/h for the multiple emulsion. This may reflect the higher water content in the double emulsion which will lower the thermodynamic activity of caffeine compared with the single emulsion. The authors did not comment on the rationale for evaluation of a large excess of the formulation in the *in vitro* study.

Dreher et al. (2002) evaluated *in vitro* skin permeation of caffeine from O/W and W/O emulsions as well as from a hydrogel under finite dose conditions. All preparations contained caffeine 1% w/w. The oil phases for the emulsions were similar and contained silicone oil and almond oil. For the O/W emulsion sorbitan tristearate and PEG-40 stearate were used as emulgents; cetyl dimethicone dipolyol and methyl glucose dioleate were used to emulsify the W/O formulation. The hydrogel consisted of water, ethanol, Carbomer®, triethanolamine, imidazolidinyl urea and methylparaben. Preparations were applied at a dose of 10 μg/cm^2 to human skin mounted in Franz cells and permeation was monitored for 24 h. At the end of the experiment skin was washed and tape stripped to remove the SC. Epidermis was then separated from the dermis by a heat treatment and tissues were digested and treated to extract caffeine. The cumulative percentage of caffeine which permeated was 15-20%. No significant differences were observed between the various formulations. About 80% of caffeine was recovered from the surface of the skin with 1% extracted from the epidermis.

Caffeine delivery from microemulsions, emulsions and hydrogel formulations was studied by Bolzinger et al. (2007). Microemulsions are composed of water, oils and and surfactants and form single phase thermodynamically stable systems (Santos et al., 2008). The emulsion and microemulsion formulations contained the same lipophilic components namely isostearyl isostearate, cyclomethicone and diisopropyl adipate and the microemulsion also contained PG. Carbomer® was used to prepare the hydrogel which also contained the same amount of PG as the microemulsion (2%). For permeation studies 1 g (equivalent to 8 mg of caffeine) of each formulation was applied to full thickness porcine skin in Franz cells with an effective diffusion area of 2.54 cm^2. Interestingly, skin samples with and without hypodermis were prepared as the authors noted that to exert lipolytic effects caffeine would need to reach the deeper adipose tissue. Cumulative amounts of caffeine which permeated were highest for the microemulsion formulation for skin samples containing hypodermis (~400 μg/cm^2) and for tissue with no hypodermis (600 μg/cm^2). Mass balance studies indicated that the highest amount of caffeine was also delivered to the hypodermis from the microemulsion formulations. Despite the higher surfactant content in the microemulsion formulations Trans epidermal water loss (TEWL) measurements indicated that the barrier function of

skin samples was not compromised following exposure to these formulations. This is the only *in vitro* study which actually examines whether caffeine permeates to the proposed target site for cosmetic effects.

Solid particles are typically used to stabilise the oil and water interface in Pickering emulsions and such systems are reported to have more favourable stability characteristics than emulsions stabilised with emulgents. Frelichowska et al. (2009) investigated Pickering W/O emulsions for caffeine delivery compared with conventional W/O emulsions. The Pickering emulsions under investigation were prepared with silica and contained the same lipophilic phase (a blend of cylomethicones) as the conventional emulsion; caffeine content was 0.8% (w/w) for both emulsions. Formulations were loaded onto full thickness porcine skin in Franz cells (0.5 g/2.54 cm^2). Cumulative amounts of caffeine which permeated after 24 h were ~500 $\mu g/cm^2$ for the Pickering emulsion and ~220 $\mu g/cm^2$ for the conventional emulsion. The more favourable penetration from the Pickering emulsion was suggested to reflect specific interactions of formulation components and/or particles with the skin. Tape stripping studies indicated the presence of silica particles in the SC and the authors also demonstrated greater adhesion energy of Pickering systems compared with the conventional emulsion. The latter was proposed to contribute to the surprisingly higher levels of permeation observed for these systems which effectively encapsulate the drug.

The influence of microemulsion structure on the skin permeation of caffeine was evaluated by Naoui and co-workers (2011). Three types of microemulsions containing caffeine were studied: O/W, W/O and bicontinuous; an aqueous solution of caffeine was included as a control (Table 5). Formulations (1 g/2.54 cm^2) containing 0.8% (w/w) caffeine were applied to full thickness porcine skin and permeation was monitored for 24 h. The cumulative permeation at 24 h for the formulations and the steady state flux values could be ranked as follows: O/W microemulsion > bicontinuous microemulsion> W/O microemulsion = caffeine solution. Permeation data were comparable or better than observed for the earlier study which employed the same experimental conditions (Bolzinger et al., 2007). Importantly, the authors also noted the possible penetration enhancement effects associated with solubilisation of skin lipids by surfactant components.

Table 5. Composition of microemulsions and aqueous solution for caffeine skin delivery studied by Naoui et al, (2010)

Component	O/W	Bicontinuous	W/O	Aqueous Solution
Tween® 21	30	27	24	-
Span® 20	-	3	6	-
Isononyl isononanoate	35	35	35	-
Water	35	35	35	100

A range of microemulsions (bicontinuous, O/W and W/O) containing caffeine (1% w/w) were also evaluated by Zhang and Michniak-Kohn (2011). Labrasol®, Cremophor® EL and isopropyl myristate were used as the oil, surfactant and co-surfactant components of the various systems; Azone® or bromo-iminosulfurane were also incorporated in some formulations as permeation enhancers. Microemulsions were applied to dermatomed porcine skin; the volume applied was 150 μL but the diffusional area of the Franz cells was not documented. Maximum cumulative amounts permeated were 240 ug/cm^2 for the O/W microemulsion; incorporation of penetration enhancers did not promote any further enhancement from this formulation.

Sintov and Greenberg (2014) prepared caffeine microemulsions (1% w/w) using isopropyl palmitate as the oil phase, Labrasol® and glyceryl oleate as surfactants, propylene carbonate and varying proportions of water. A microemulsion gel containing caffeine was also prepared by incorporation of amorphous silica into the formulation containing 20 % water. Formulations (0.5 g) were applied to full thickness porcine skin (diffusional area 1.767 cm^2) which had been freshly prepared or frozen (-20°C) and an aqueous solution of caffeine was used as the control. The maximum amount of caffeine permeation at 24 h from the aqueous solution was ~80 μg/cm^2 for the freshly prepared tissue. For the W/O liquid emulsion and for the microemulsion gel containing 20% water the corresponding amounts permeated were 320 μg/cm^2 and 270 μg/cm^2. Permeation data for the frozen tissue was much higher which the authors attributed to storage however this problem has not been reported previously.

4.4 Nanoparticles

Nanoparticles prepared with starch derivatives and loaded with caffeine were prepared by Santander-Ortega et al. (2010). Permeation experiments conducted with human skin indicated <0.2

μg/cm^2 of caffeine permeated after 12 h in Franz cells. Surprisingly the authors stated that the data suggested the potential of these carriers for transdermal delivery. Silica-caffeine nano-composites were prepared using a ball-milling process by Pilloni and co-workers (2013). Subsequently a gel formulation containing the nano-composites as well as a control gel formulation were evaluated *in vitro* using new-born pig skin. Caffeine loading in both formulations was 3 % (w/w) and formulations were dosed to skin samples at either 100 or 200 mg to an effective diffusional area of 0.6 cm^2. After 8 h permeation the amounts of caffeine permeated were markedly lower for the nano-composite formulation (~100 μg and ~180 μg) compared with the control formulation (~500 μg and 600 μg). Washing and mass balance studies suggested that most of the drug in the nano-composite formulation had remained on the surface of the skin.

Puglia et al. (2014) prepared solid lipid nanoparticles (SLN) loaded with caffeine(SLN-CAF) by solvent emulsification and dispersion. The SLN-CAF dispersion was then loaded into a xanthan gel formulation and evaluated alongside a reference gel containing the same amount of caffeine. Franz cell studies were carried out with heat-separated human epidermis for 24 h. Approximately 8 μg of caffeine had permeated from the SLN-CAF gel at the end of the permeation study compared with ~3 μg from the reference gel. As the precise loading of each formulation in the cells was not provided, it is difficult to determine whether the SLN-CAF formulation is advantageous compared with other approaches.

The results for all chemical enhancement approaches assessed *in vitro* are summarised in Table 6 along with values for permeation parameters, where reported. As different concentrations are reported by different research groups caution should be exercised when comparing results for different studies. Typically, high amounts of caffeine permeation are associated with infinite dose conditions; only a limited number of studies have been conducted with finite dose application.

5. Physical enhancement methods

5.1 Mechanical pressure

Treffel and co-workers (1993) investigated the effects of pressure on caffeine permeation *in vitro*. Caffeine solutions were applied (50 μl of an acetone solution, 320 μg/ml or 15 mg/ml) to human skin samples mounted in Franz cells with an effective diffusion area of 3.14 cm^2. Following evaporation of the acetone pressure was applied using an air pump for 30 min to achieve an increase of 0.25 bar in atmospheric pressure. At the end of the 24 h permeation experiment tissue integrity was evaluated by application of methylene blue to the skin samples. No significant increase in caffeine permeation with increased pressure was observed for the lower dose however a 1.8 fold increase ($p<0.05$) in caffeine cumulative absorption was achieved for the higher dose. Actual

cumulative amounts of caffeine permeated were 9.4 ± 2.2 μg/cm^2 for the control and 17.0 ± 3.4 μg/cm^2 for the experiments conducted with application of increased pressure. The authors suggested that the higher permeation might reflect increased filling of the SC under pressure and/or a greater role for trans-appendageal transport.

5.2 Ultrasound

The application of ultrasound or sonophoresis to enhance skin permeation of caffeine was reported by Mitragotri et al. (1994). Heat separated human skin was mounted in diffusion cells which were modified to accommodate an ultrasound transducer. Experiments were conducted at room temperature and a thermocouple was used to monitor any variation in temperature over the course of the experiment. Radiolabelled caffeine was applied in the donor compartment (total volume 8.0 mL) at a concentration of 1 μCi/mL in PBS to an effective diffusion area of 3.14 cm^2. An ultrasound generator was used to apply continuous ultrasound at frequencies of 1 or 3 MHz with intensities up to 2 W/cm^2. Tissue resistance was monitored at regular intervals before, during and after ultrasound treatment. Only modest improvements in caffeine permeation (1.2 fold enhancement) were reported compared with the control. This may reflect the temperature rise which was observed after the initial application of ultrasound. Continuous and pulsed ultrasound was used to promote caffeine delivery in human skin *in vitro* by Boucaud et al. (2001). A solution of radiolabelled caffeine (5 μCi/mL) was applied to the donor compartment at a dose of 3 mL/3.14 cm^2. Ultrasound was applied at a frequency of 20 Hz and an intensity of 2.5 W/m^2. Although the amount of caffeine which permeated after 8 h increased for both continuous (~35 ng/cm^2) and pulsed (~25 ng/cm^2) treatments compared with the control (~10 ng/cm^2), a temperature increase was also associated with the ultrasound application.

An *in vivo* study using pigs was conducted to investigate the influence of ultrasound on caffeine permeation and skin morphology by Pires-de-Campos et al. (2008). Five treatment sites were delineated on shaved dorsal areas of the animals. Treatments were as follows: placebo gel, gel containing caffeine (5 % w/w), gel and ulstrasound, gel containing caffeine and ultrasound. Continuous ultrasound was used for 1 min/cm^2 at a frequency of 3 MHz and an intensity of 0.2 W/cm^2. Histological processing confirmed that sites treated with ultrasound and caffeine were thinner than other treatment sites; a reduction in the number of adipocytes was also claimed however caffeine content of the tissue was not determined.

5.3 Iontophoresis

Iontophoresis as an approach to enhance skin penetration of caffeine has been studied by Marra and colleagues (2008). A device which combined a low voltage to generate a constant current along with pulses of high-frequency electro-porating voltage was employed. A simple gel was prepared containing water, PG and a gelling agent; a second gel containing caffeine and a number of other components was then added to this base gel. This was then applied to porcine ear skin mounted in vertical diffusion cells. A set of silver/silver chloride electrodes in the receptor chamber completed the electrical circuit and the current was monitored throughout the experiment. The intensity setting applied was 6 V and frequency and duration of voltage pulses was 1724 Hz and 160 μsec respectively. The total electro-treatment time was 20 min and passive permeation was also investigated as a control. Significantly greater ($p<0.05$) amounts of caffeine were determined in tissue for electro-treatment compared with passive diffusion for 20 min. No significant differences in tissue levels of caffeine were observed for electro-treatment for 20 min followed by 60 min passive diffusion compared with 80 min passive diffusion.

5.4 Microneedles

Donnelly and co-workers have pioneered the development and application of drug loaded dissolving microneedles (MNs) for topical and transdermal drug delivery (Donnelly et al., 2008; Migalska et al., 2011). MNs prepared from blends of poly (methyl vinyl ether co maleic acid) were loaded with caffeine (3.1 mg) and evaluated following application to dermatomed (300 -350 μm) and full thickness (700 -750 μm) neonatal porcine skin in Franz cells (2012). Control patches loaded with caffeine but without MNs were also investigated for comparison. After 24 h the cumulative amounts of caffeine which permeated from the control and MN patches were respectively 136.4 ± 23.2 μg and 1833.8 ± 302.3 μg for dermatomed skin and 81.4 ± 15.2 μg and 1408.6 ± 133.7 μg for full thickness skin. Optical coherence tomography indicated that MN penetrated into the skin to a depth of 430 μm creating a conduit or pore of ~220 μm.

6. Summary and conclusions

Considerable efforts have been made in recent decades and are still ongoing to promote dermal delivery of caffeine. The literature evaluated reports studies conducted with relatively simple vehicles as well as more complex preparations. Of the various passive formulation strategies to promote percutaneous penetration of caffeine which have been reviewed, the greatest effort has centred on either conventional emulsions or microemulsions. A problem with many of these studies is the use of infinite dose conditions which over-estimate the penetration enhancement which will

be achieved for more realistic finite dose application conditions. Although some commercial cosmetic patch formulations are available which likely do contain infinite does of caffeine, data on their efficacy and delivery characteristics are lacking. Overall, the results for the various permeation studies suggest that a large percentage of the applied caffeine resides on the skin surface. In order to optimise formulations in the future it will be necessary to identify strategies which address this problem. The caffeine needs to remain in solution in order to permeate, the caffeine needs to be at a high thermodynamic activity and also not crystallise in the SC once it has partitioned into the skin lipids. Until recently transdermal delivery of caffeine has only been feasible for premature infants because of their compromised skin barrier. The advent of MNs has expanded the possibilities for systemic delivery of caffeine. Caffeine has a long history as a model hydrophilic compound thus the data collated here should have relevance for other molecules with similar physicochemical properties.

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