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Synergistic Skin Penetration Enhancer and Nanoemulsion Formulations Promote the Human Epidermal Permeation of Caffeine and Naproxen

Eman Abd [1, 2], Sarika Namjoshi [1], Yousuf H. Mohammed [1], Michael S. Roberts [1, 3, *], Jeffrey E. Grice [1]

[1] Therapeutics Research Centre, School of Medicine, University of Queensland, Translational Research Institute, Brisbane, Australia
[2] College of Health and Medical Technology, Baghdad, Iraq
[3] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, Australia

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ABSTRACT

We examined the extent of skin permeation enhancement of the hydrophilic drug caffeine and lipophilic drug naproxen applied in nanoemulsions incorporating skin penetration enhancers. Infinite doses of fully characterized oil-in-water nanoemulsions containing the skin penetration enhancers oleic acid or eucalyptol as oil phases and caffeine (3%) or naproxen (2%) were applied to human epidermal membranes in Franz diffusion cells, along with aqueous control solutions. Caffeine and naproxen fluxes were determined over 8 h. Solute solubility in the formulations and in the stratum corneum (SC), as well as the uptake of product components into the SC were measured. The nanoemulsions significantly enhanced the skin penetration of caffeine and naproxen, compared to aqueous control solutions. Caffeine maximum flux enhancement was associated with a synergistic increase in both caffeine SC solubility and skin diffusivity, whereas a formulation-increased solubility in the SC was the dominant determinant for increased naproxen fluxes. Enhancements in SC solubility were related to the uptake of the formulation excipients containing the active compounds into the SC. Enhanced skin penetration in these systems is largely driven by uptake of formulation excipients containing the active compounds into the SC with impacts on SC solubility and diffusivity.

Introduction

Percutaneous absorption offers an attractive noninvasive route of administration for local topical or systemic effects but is limited by the skin's inherent barrier to penetration of any exogenous material. It is well established that the uppermost layer of the skin, the stratum corneum (SC) is the main barrier to such penetration but can be overcome to meet therapeutic and cosmetic goals by prudent considerations of the active's potency, physicochemical properties, formulation, and delivery systems.[1] Formulation approaches include optimization, use of prodrugs, and incorporation of chemical or biological modifiers to transiently reduce SC barrier function. The range of delivery systems in current use includes: topical products, transdermal patches, physical methods such as microneedles and heat as well as other technologies, including iontophoresis, sonophoresis, radiofrequency, and laser ablation.[1]

Microemulsions and nanoemulsions, defined as single phase and thermodynamically stable isotropic systems composed of water, oil, and amphiphilic molecules,[2] are attractive systems for enhancing drug delivery to the skin because of their ease of formulation, thermodynamic stability, and solubilization.[3] They are capable of incorporating and enhancing the skin delivery of both hydrophilic and lipophilic drugs[4,5] and are considered to be more stable than conventional emulsions because of the small droplet sizes preventing phase separation. Moreover, small droplet sizes provide better adherence to membranes, leading to more efficient transport of drug molecules in a controlled fashion.[6,7]

Microemulsions and nanoemulsions may be categorized into three main types: water in oil (w/o), bicontinuous, and oil in water (o/w), though a mixture of oil, water, and surfactants will be able to generate a variety of structures and phases.[5,8,9] When similar amounts of oil and water are used, the structures formed are not well characterized and are assumed to be continuous.[10] Although microemulsion and nanoemulsion formation depends on the capacity of the surfactant system to decrease the surface tension, in

* *Correspondence to:* Michael S. Roberts (Telephone: +61-7-34438032).
E-mail address: m.roberts@uq.edu.au (M.S. Roberts).

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practice almost all surfactants require the presence of additional cosurfactants. Excipients such as short- or long-chain alcohols or polyglycerol derivatives have been used to achieve low surface tension. Addition of a cosurfactant reduces the interfacial tension as well as the critical micelle concentration. The correct selection of components is the main factor to be considered when formulating microemulsions for topical or transdermal delivery.[11] Microemulsions and nanoemulsions may enhance topical and transdermal delivery mainly by increasing the solubilization capacity for hydrophilic and lipophilic compounds, maintaining constant supply of the drug from the internal to the external phase and thus keeping the external phase saturated and promoting skin absorption. The formulation ingredients such as the oil, surfactants, cosurfactants, and penetration enhancers may increase drug diffusion by enhancing partitioning through the skin. Also, the low interfacial tension required for microemulsion and nanoemulsion formation may be responsible for the excellent wetting properties, which ensures surface contact between the membrane and the vehicle.[12] In addition to their favorable permeation enhancement properties, microemulsions and nanoemulsions may also reduce skin irritancy of certain excipients. For example, an aqueous solution containing 20% propylene glycol was shown to cause irritation, but the same concentration of propylene glycol used as a cosurfactant in microemulsion formulations did not.[13]

The components of the oil phase in a microemulsion may include penetration enhancers such as lecithin, hydrophilic terpenes such as eucalyptol (EU), or unsaturated fatty acids such as oleic acid (OA) to enhance the permeation of the active through the skin without causing local irritation.[14,15] Studies suggest that these penetration enhancers may cause disruption of the SC lipid organization, thus increasing the fluidity and decreasing diffusion resistance to solutes.[14,16]

The objective of this study was to investigate the synergy of including skin penetration enhancers in nanoemulsions on human epidermal permeation for a model hydrophilic compound (caffeine; log P, −0.07) and a lipophilic compound (naproxen; log P, 3.18). OA and EU were the penetration enhancers studied. Each formulation was characterized in terms of its physical and chemical properties, including deriving their apparent solubility parameters. We then carried out *in vitro* human epidermal permeability studies in Franz diffusion cells and evaluated the permeation of caffeine and naproxen from nanoemulsions, formulated with skin penetration enhancers as the oil phase, and various control solutions. As described in our previous work,[17,18] we also estimated for each active its saturated flux, solubility in the SC, and diffusivity as well as quantifying the extent of formulation uptake into the SC. These were then used to investigate the mechanism by which the nanoemulsions facilitated an enhanced permeation of active across the human epidermis.

Materials and Methods

Chemicals

Caffeine, naproxen, ethanol, OA, and EU were purchased from Sigma-Aldrich Pty. Ltd. (Sydney, NSW, Australia). Volpo-N10 was obtained from Umiqema (Witton Centre, Witton Redcar TS10 4RF, UK). All chromatography reagents were analytical reagent grade.

Preparation of Emulsions

Volpo-N10 (an ethoxylated fatty alcohol, also known as Oleth-10 or Brij96v, acting as a nonionic surfactant) was dissolved in ethanol (cosurfactant) in a 1:1 ratio. The resulting mixture was then mixed with the oil phase, OA or EU (oil), in a 0.6:1:1 ratio followed by gentle mixing with phosphate-buffered saline (PBS). The resulting nanoemulsion was clear at room temperature. Caffeine and naproxen were dissolved in the nanoemulsions and control solutions at 3% (w/w) and 2% (w/w), respectively. A pseudo-ternary phase diagram was constructed using the water titration method. At the weight ratio of 1:1, the highest amount of water was solubilized in the system. The O/(S/Co-S) mixture was diluted drop wise with PBS under moderate agitation. The samples were classified as nanoemulsions when they appeared as clear liquids. The compositions of emulsions and control solutions made are shown in Table 1.

The procedure for preparing emulsions is summarized as follows:

1. Volpo-N10 (surfactant, S) dissolved in ethanol (cosurfactant, Co-S) in 1:1 ratio
2. The S:Co-S mixture mixed with oil phase (OA or EU) in a 0.6:1:1 ratio
3. PBS added to the mixture with gentle mixing
4. Caffeine or naproxen dissolved in nanoemulsions at 3% and 2% (w/w), respectively

Characterization of Emulsions

The droplet size distributions, refractive indices, and electrical conductivities of the emulsions were determined at ambient temperature using dynamic light scattering (Zetasizer Nano ZS; Malvern Instruments, Ltd., Malvern, UK), an RFM34 refractometer (Bellingham & Stanley, Tunbridge Wells, Kent, UK) and a Digitor Multimeter (DSE Limited, Sydney, NSW, Australia), respectively. Electrical conductivity measurement enables identification of the continuous phase of the emulsion, with o/w emulsions being conductive, whereas w/o emulsions are not. The viscosity of the emulsion formulations was measured using a U-tube viscometer at 25°C. All determinations were performed with three replicates.

Human Skin Preparation

Skin samples were obtained with informed consent from female patients undergoing elective abdominoplasty, and approval from the University of Queensland Human Research Ethics Committee (HREC Approval no. 2008001342). The procedures were conducted in compliance with guidelines of the National Health and Medical Research Council of Australia. Full thickness skin was prepared by removal of subcutaneous fat by blunt dissection. Heat separation was used to separate epidermal membranes from full thickness skin, by immersing it in water at 60°C for 1 min, to allow the epidermis to be teased away from the dermis.[19] SC was prepared from the epidermal membranes by trypsin digestion.[20] The

Table 1
Compositions (%, w/w) of Control Solutions (C1-C4) and Nanoemulsion Formulations With Penetration Enhancers Eucalyptol (E1 and E2) and Oleic Acid (O1 and O2)

Variable	Water	Ethanol	PEG-6000	Volpo-N10	Eucalyptol	Oleic Acid
$C1^{C}$	100	–	–	–	–	–
$C2^{C,N}$	40	60	–	–	–	–
$C3^{C}$	75	–	25	–	–	–
$C4^{N}$	50	25	–	25	–	–
$E1^{C,N}$	30.97	26.55	–	26.55	15.93	–
$E2^{C,N}$	36.59	24.39	–	24.39	14.63	–
$O1^{C,N}$	30.97	26.55	–	26.55	–	15.93
$O2^{C,N}$	36.59	24.39	–	24.39	–	14.63

The concentration of caffeine (marked in superscript C) dissolved in aqueous controls and nanoemulsions was 3% (w/w), whereas a concentration of 2% (w/w) was used for naproxen (marked in superscript N).

epidermis was floated overnight on a solution of 0.01% trypsin in phosphate buffer saline at 37°C. The digested viable epidermis was gently scraped off with cotton buds and the remaining SC membrane was rinsed several times with distilled water. The isolated SC membranes were dried with absorbent paper and placed flat between parafilm sheets covered with aluminum foil. All skin membranes were stored frozen at −20°C until use.

Determination of the Solubility of Actives in the Various Formulations

The solubility of caffeine and naproxen in each formulation (S_V) was determined by adding caffeine or naproxen to 5 mL of each nanoemulsion or control solution until an excess amount remained. The samples were then incubated in a water bath at 32°C for 24 h with continuous agitation and centrifuged at 4300 g for 10 min. The supernatant was withdrawn and diluted to accurately quantify the amount of each compound by HPLC.

Determination of Solubility in the SC and Solvent Uptake

To determine the SC solubilities of caffeine and naproxen from the various vehicles, preweighed discs of SC (four replicates for each compound) were incubated in 1 mL saturated solutions of each compound in the various vehicles at 32°C for 24 h.[20] At the end of the incubation period, the SC discs were removed and blotted dry. The SC was further incubated with 1 mL of 70% ethanol–water for 24 h at 32°C to enable complete extraction of the solutes. S_{SC} was determined from the amount recovered in the extraction fluid measured by HPLC divided by the thickness and area of the SC.[17] The total solvent uptake into the SC was determined from the weight differences of the dry pieces of the SC (about 0.7 mg) soaked in each formulation (1 mL) for 24 h at 32°C. The SC pieces wiped three times with Kimwipe tissues before weighing again.

In Vitro *Skin Permeation Study*

In vitro skin permeation studies were performed with epidermal membranes in Franz diffusion cells with an effective diffusion area of 1.33 cm^2 and approximately 3.4 mL receptor chamber capacity. The skin was cut into discs and mounted between the donor and receptor compartment of the Franz cell with the SC side facing the donor chamber. The receptor compartment containing PBS (pH 7.4) was immersed in a water bath at 35 ± 0.5°C. The donor solution consisted of 1 mL of the nanoemulsion or control formulations, containing either 3% (w/w) caffeine or 2% (w/w) naproxen. The donor compartment was covered with parafilm to prevent evaporation. At predetermined time points, 200 μL of the receptor phase was withdrawn and replaced with an equal volume of fresh PBS. The caffeine and naproxen content in all samples was determined by HPLC.

HPLC Analysis of Caffeine and Naproxen

Caffeine and naproxen in solutions and extracts from various matrices were analyzed by a sensitive and rapid HPLC method. The HPLC system consisted of a Shimadzu SIL-20 a HT, CBM-20A system controller, a SPD-20A detector, a LC-20AD pump, and an auto injector (Shimadzu Corp., Kyoto, Japan). Isocratic separation of both caffeine and naproxen was achieved on Phenomenex Luna 5 μm, C18 (150 × 4.6 mm) column (Phenomenex Inc., Torrance, CA). For caffeine analysis, elution was performed at ambient temperature with a mobile phase of 95% water, 2% acetonitrile, 2% tetrahydrofuran, and 0.5% acetic acid at a flow rate of 1 mL/min. Detection wavelength was 273 nm. For naproxen, the mobile phase consisted of 35% water, 45% acetonitrile, 20% methanol, and 0.3% acetic acid at a flow rate of 0.7 mL/min. The detection wavelength was 230 nm.

Data Analysis

The cumulative amount (Q, μg/cm^2) of caffeine and naproxen penetrating through an area of 1.3 cm^2 was plotted against time (t). The steady-state flux J_{SS} (μg/cm^2h) was determined from the slope of the linear portion of the cumulative amount (Q) versus time t plot.

The maximum flux (J_{max}) that would be applicable to saturated solutions can be estimated from the experimental steady-state flux corrected for the known solubility in the formulation by Eq. (2)[17]:

$$J_{max} = J_{SS} S_V / C_V \quad (1)$$

where S_V is the solubility in the formulation and C_V is the experimental concentration used.

The apparent diffusivity of solute in the skin divided by path length (D*) was calculated from the maximum flux and the solubility of the active compound in the SC according to Eq. (2).[17]

$$D^* = J_{max} / S_{SC} \quad (2)$$

where S_{SC} is the experimentally determined solubility of the solute in the SC.

Hansen solubility parameters (δ_D, δ_P, δ_H) for formulation excipients, solvents, and active compounds were obtained from the software package HSPiP (JW Solutions B.V., Gouda, The Netherlands). The total solubility parameters were also obtained from HSPiP, according to the formula:

$$\delta^2_{total} = \delta^2_D + \delta^2_P + \delta^2_H \quad (3)$$

Values of the solubility parameters for human skin ($\delta_D = 17$, $\delta_P = 8$, and $\delta_H = 8$) were taken from Hansen[21] and used with the reported solubility parameters for caffeine ($\delta_D = 19.5$, $\delta_P = 10.1$, and $\delta_H = 13.0$), naproxen ($\delta_D = 18.9$, $\delta_P = 4.3$, and $\delta_H = 9.9$) to estimate the HSP distance (Ra), a measure of the similarity between two materials:

$$Ra = \left[4(\delta_{D1} - \delta_{D2})^2 + (\delta_{P1} - \delta_{P2})^2 + (\delta_{H1} - \delta_{H2})^2\right]^{1/2} \quad (4)$$

Statistics

All experiments were analyzed by one-way analysis of variance (ANOVA) with post-hoc comparisons (Tukey) using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA). p value less than 0.05 was considered significant.

Comparisons were made between the nanoemulsion formulations, without and with enhancers, and control, as well as between the different nanoemulsion formulations for the cumulative amount permeated at different time points and the amount of caffeine and naproxen recovered following skin extraction. A result was considered significant when p was less than 0.05.

Results

Physical and Chemical Characterisation of the Formulations Used

Table 2 summarizes the physical and chemical properties of the various formulations used in this study. In general, all formulations

Table 2
Physical and Chemical Characterisation of the Various Control Solutions and Micoemulsion Formulations Defined in Table 1

Property	Control Solutions (C1-C4)				Nanoemulsions + Eucalyptol (E1, E2)		Nanoemulsions + Oleic Acid (O1, O2)	
	$C1^{C}$	$C2^{C,N}$	$C3^{C}$	$C4^{N}$	$E1^{C,N}$	$E2^{C,N}$	$O1^{C,N}$	$O2^{C,N}$
Appearance	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear
Viscosity (cp)	0.96	2.65	22.20	25.40	13.7 ± 4.5	15.1 ± 4.0	23.0 ± 4.7	28.3 ± 4.5
Conductivity (μS)	0.21	1.15	0.12	1.05	87.5 ± 2.2	91.3 ± 3.9	80.8 ± 8.2	84.5 ± 10.1
Refractive index	1.33	1.36	1.32	1.34	1.38	1.37	1.38	1.37
Droplet size (nm) (emulsion only)	–	–	–	–	29.6 ± 3.1	19.5 ± 1.3	8.0 ± 0.5	12.4 ± 0.1
Droplet size (nm) (emulsion + caffeine)	–	–	–	–	19.3 ± 4.0	16.0 ± 3.6	5.9 ± 2.4	1.2 ± 0.1
Droplet size (nm) (emulsion + naproxen)	–	–	–	–	37.8 ± 5.9	25.0 ± 3.0	11.6 ± 3.8	13.5 ± 4.5
Hansen solubility parameters $(MPa)^{1/2}$								
δ_D	15.5	15.4	16.7	15	15.3	15.3	15.3	15.3
δ_P	16	11.9	13.6	11.9	9.4	9.3	9.4	10
δ_H	42.3	28.7	25.7	28.4	20.7	22.5	21.2	22.9
δ_{Total}	47.8	34.7	33.5	34.3	27.4	28.8	27.8	29.3
(HSP dist)[a]	43.2	26.3	29.1	20.7	15.8	17.3	14.8	16.8
Molar volume (MV)	18	30.8	35.9	30.9	43.3	38.9	44.2	39.5

[a] HSP distances (Ra) of mixtures calculated from Eq. (4), using software package HSPiP.

were clear and have a low viscosity. As one example, shown in Figure 1, the nanoemulsion area in the pseudo-ternary phase diagram with a 1:1 (w/w) ratio of Volpo-N10 to ethanol is in the region with a surfactant concentration between 80% and 40%. In this region, a high amount of water can be solubilized without causing phase separation and is characterized by transparency and higher viscosity. In contrast, in other regions of the phase diagram, a turbid emulsion is seen. Figure 1 also shows that the proportion of the ternary diagram existing as a nanoemulsion increases as the amount of water increases and the concentration of surfactant/cosurfactant mixture decreases.

Table 2 also shows the viscosity, conductivity, refractive index and nanoemulsion droplet size. In general, these are similar. However, it is apparent that consistent with OA's surface-active properties, the droplets from the nanoemulsions containing OA are smaller than with EU. In addition, droplet sizes of emulsions containing caffeine are generally smaller than the corresponding ones containing naproxen, also consistent with the known surface active and self-aggregation properties of caffeine.[22] Further, all nanoemulsions had mean droplet sizes less than 100 nm, indicating that they should be regarded as being nanoemulsions, and high conductivies, consistent with them being oil in water (o/w) nanoemulsions. Also included in Table 2 are the estimated solubility parameters and "average" molar volume for each of the formulations studied. It is evident that the various solubility parameters are in the general order: water> nanoemulsions>nanoemulsions containing penetration enhancers.

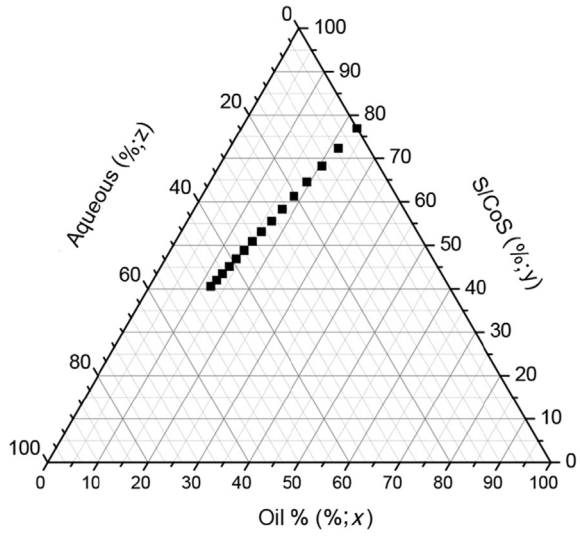

Figure 1. Ternary phase diagram of the oil, surfactant–cosurfactant mixture, and water at ambient temperature. The dotted line in the diagram represents the nanoemulsion area.

In Vitro *Permeation of Caffeine and Naproxen Across Epidermal Membranes*

Figure 2a shows the cumulative amount (μg/cm^2) of caffeine penetrated across epidermal membranes versus time for nanoemulsions and controls over the duration of the study. Penetration from nanoemulsions containing skin penetration enhancers was greater than from control vehicles. The corresponding cumulative amount versus time profiles for naproxen are shown in Figure 2b, where similarly greater penetration occurs from nanoemulsions compared with controls. For both caffeine and naproxen, the ethanol–water solution promoted a greater epidermal penetration than the other control solutions.

Impact of Formulation on Active Solubility in Formulation, Solubility in SC, Maximum Flux, and Derived Diffusivity

Table 3 shows the estimated active solubility in formulation, solubility in SC, maximum flux calculated from steady-state flux using Eq. (1), and diffusivity per path length D^* derived from the maximum flux and SC solubility using Eq. (2). It is apparent that both caffeine and naproxen have a much higher solubility in the nanoemulsion formulations containing enhancers than in controls. Indeed, the solubility of caffeine in water was much less than the corresponding values with the other controls and with the emulsions, and naproxen had a higher solubility in the nanoemulsions than in the controls. Pronounced enhancements in caffeine and naproxen maximum fluxes were evident for the aqueous ethanolic solution and the various emulsion formulations containing enhancers, the enhancement being between one and two orders of magnitude greater than the controls (Fig. 3). It is apparent that the solubility of caffeine in the SC after application of various emulsion formulations containing enhancers is modest, whereas the derived D^* for these formulations increases by one or two orders of magnitude. It is thus apparent that the marked formulation enhancement in caffeine penetration arises from the enhancers reducing the diffusion resistance for caffeine permeation. In contrast, the solubility of naproxen in the SC after application of

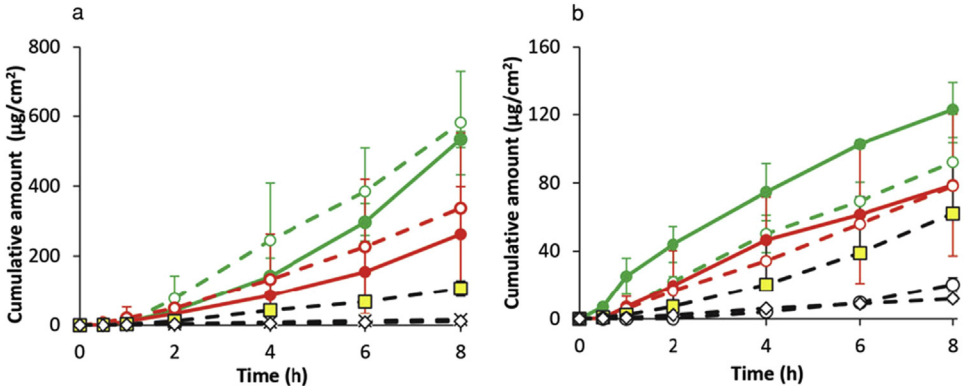

Figure 2. *In vitro* percutaneous permeation through epidermal human skin: (a) caffeine; (b) naproxen (E1, green solid lines; E2, green dashed lines; O1, red solid lines; O2, red dashed lines). C1 (water), crosses; C2 (60% EtOH/water), yellow squares; C3 (50% PEG6000/water), diamonds; and C4 (Volpo-N10/EtOH/water), circles.

various emulsion formulations containing enhancers is about an order of magnitude greater than the controls, whereas the derived D^* is not greatly different from the slight enhanced D^* seen for an aqueous ethanolic formulation.

The different mechanisms of enhancement for the nanoemulsions with enhancers for caffeine and naproxen are more clearly shown in Figure 4. In Figure 4a, it is evident that the J_{max} for naproxen is correlated with its solubility in the SC, whereas as shown in Figure 4b, caffeine is better related to its altered diffusivity D^* associated with various formulations.

Mechanism Underpinning the Differential Enhanced Uptake of Caffeine and Naproxen into the Epidermis

Figure 5 shows that the enhanced solubility in the SC from various formulations could be related to the uptake of both caffeine and vehicle with the vehicle into the SC. An optimal regression was found when it was assumed that all the dissolved solute in the vehicle was taken up into the SC as defined by:

$$S_{SC}(\text{predicted}) = S_{SC}(\text{skin, control}) + S_V(\text{vehicle}) * V_{up}/V_{SC}$$

where V_{up} is solvent uptake and V_{SC} is the volume of the SC. S_{SC} (skin, control) represents the solute in the SC, derived from the experimental data for a control solution:

$$S_{SC}(\text{skin, control}) = S_{SC}(\text{exp, control}) = S_f(\text{exp, control}) * V_{up}(\text{control})/V_{SC}$$

An excellent S_{SC} (predicted) versus S_{SC} (experimental) correlation is evident for naproxen ($R^2 = 0.84$) but not for caffeine ($R^2 = 0.48$). The deviation from linearity for caffeine in the nanoemulsions could be because of its high solubility in these formulations, with a corresponding increase in predicted SC solubility.

Can the Solubility of the Actives in Various Formulations and the Uptake of Formulations Into SC Be Predicted by Solubility Parameter Approach?

Figure 6 shows the relationships between the solubility of solutes in the formulations and the uptake of formulations into the SC as functions of the HSP distance (Ra). Figure 6a shows that the experimental solubilities of caffeine and naproxen in the various vehicles is highest when Ra is lowest and when the solutes and nanoemulsions are most similar in their various solubility parameters. Figure 6b shows that solvent uptake into the SC is also greatest for those formulations when Ra is lowest, that is, formulations that are most similar to the SC in their various solubility parameters.

Discussion

This work has demonstrated that the skin permeation of caffeine and naproxen is markedly enhanced using nanoemulsion formulations containing the penetration enhancers OA and EU, in comparison to all controls. These results are consistent with those previously reported for the skin permeation of hydrophilic and lipophilic drugs using microemulsion formulations.[23] A key question we also addressed was how the nanoemulsions with the penetration enhancers promoted skin penetration. It is evident that the nanoemulsions made in this study worked by three

Table 3
Experimental Data for Caffeine and Naproxen in Different Nanoemulsions Without and With Penetration Enhancers and Control Vehicles

Formulation	Active							
	Caffeine				Naproxen			
	S_V (mg/mL)	J_{max} (µg/cm²h)	S_{SC} (µg/mL)	D* (cm/h *1E4)	S_V (mg/mL)	J_{max} (µg/cm²h)	S_{SC} (µg/mL)	D* (cm/h *1E4)
C1	31.3 ± 1.2	2.2 ± 0.8	21.6 ± 2.2	1.0 ± 0.4	–	–	–	–
C2	81.5 ± 13.4	25.6 ± 3.1	151.0 ± 37.7	1.7 ± 0.2	50.9 ± 1.4	23.4 ± 4.8	18.3 ± 6.1	12.8 ± 2.6
C3	57.4 ± 10.1	2.5 ± 0.7	49.4 ± 14.2	0.5 ± 0.1	66.8 ± 1.9	6.2 ± 0.3	16.0 ± 1.8	3.9 ± 0.2
C4	–	–	–	–	25.9 ± 1.5	7.3 ± 2.7	9.5 ± 1.7	7.7 ± 2.9
E1	112.3 ± 8.5	263.6 ± 1.2	38.2 ± 15.3	69.1 ± 0.3	174.9 ± 8.3	122.4 ± 27.1	101.8 ± 22.4	12.0 ± 2.7
E2	110.8 ± 9.5	267.7 ± 24.0	36.5 ± 4.1	73.3 ± 6.6	148.1 ± 5.1	86.6 ± 8.9	114.0 ± 35.1	7.6 ± 0.8
O1	101.8 ± 2.2	118.8 ± 57.3	52.4 ± 3.9	22.7 ± 11.0	198.5 ± 0.4	101.2 ± 41.7	113.8 ± 66.7	8.9 ± 3.7
O2	90.2 ± 8.1	136.4 ± 95.2	64.4 ± 20.6	21.2 ± 14.8	170.6 ± 3.3	74.0 ± 2.3	95.9 ± 58.2	7.7 ± 0.2

Mean ± SD.
The formulations are defined in Table 1 and are nanoemulsion formulations (C3 and C4), with penetration enhancers eucalyptol (E1 and E2) and oleic acid (O1 and O2), and control mixtures (C1 and C2).

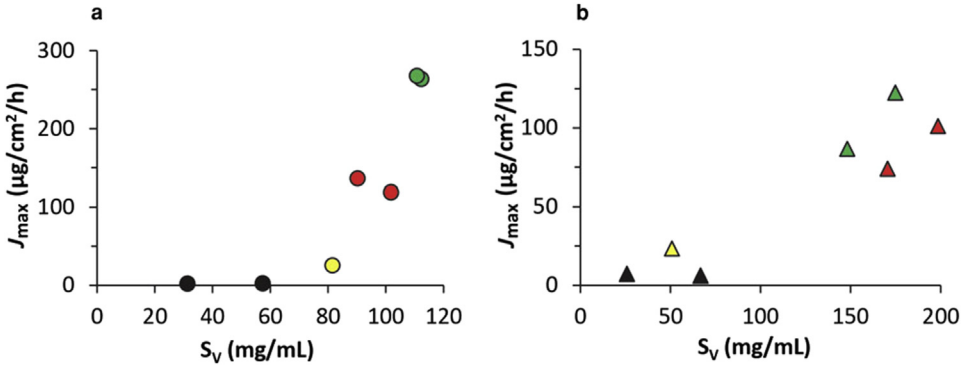

Figure 3. Estimated maximum fluxes for caffeine and naproxen for each of the formulations plotted against the solubility of these actives in the formulations. Circles, caffeine. Triangles, naproxen. Black symbols, controls (solutions in water and 25% PEG6000/water for caffeine, 25% PEG6000/water, and Volpo-N10/EtOH/water for naproxen). Yellow symbols, 60% EtOH–water. Red symbols, nanoemulsions containing OA. Green symbols, nanoemulsions containing EU.

mechanisms, which may or may not be working in concert to give a synergistic effect: (1) an enhanced solubility of the drug in the applied vehicle, (2) uptake of the vehicle carrying the caffeine and naproxen into the SC, and (3) alteration of the properties of the SC membrane, for example, by fluidisation of the SC lipids.

The combinations of surfactants and oils used in our emulsions enabled the vehicle solubility of both caffeine and naproxen to be increased above that seen with any single solvent solution or solvent mixture used in this study as controls (Table 3). The threefold to fourfold increase in solubility of the water-soluble compound caffeine in the nanoemulsions compared with the aqueous solution is most likely reflecting the process of micellar solubilisation.[24] The nanoemulsion-induced enhanced solubility was even more evident with the lipophilic naproxen, with a sixfold to eightfold solubility enhancement seen with the nanoemulsions compared with a complex cosolvent mixture containing Volpo-N10 and ethanol (25.9 mg/mL). The findings are consistent with the general view that solubilization is even more pronounced for lipophilic compounds.[25] The nanoemulsions were also associated with excellent SC solubility, particularly in the case of the more lipophilic naproxen (Table 3). Further, both EU and OA are known to disrupt the SC by multiple mechanisms, including dissolution of SC lipids.[26]

To clarify that there had been a nanoemulsion-induced permeation enhancement for caffeine and naproxen, we estimated the solute maximum (or saturated) fluxes (J_{max}). The use of maximum flux enables the potential enhancement caused by the nanoemulsions to be defined as it is now well recognized that maximum flux is independent of the vehicle and dependent solely on the thermodynamic activity of the solute in the vehicle, provided the vehicle or solute does not alter the properties of the membrane.[17,27] This concept was initially highlighted by the work of Twist and Zatz,[28] who showed the same flux for methyl paraben across synthetic membranes, regardless of its solubility in a range of different vehicles. Our results for the control solutions are consistent with these findings in that a similar low value for J_{max} for both caffeine and naproxen was found for each control solutions not affecting skin permeability across a range of vehicle solubilities. In contrast, the J_{max} values seen with nanoemulsions and to some extent ethanol–water are much higher than the controls (Fig. 3), consistent with their enhancement of skin permeability and suggesting that these vehicles have altered the properties of the stratum corenum. The penetration enhancer, EU, had a much greater effect on J_{max} than OA for the hydrophilic drug caffeine, a differentiation not seen with the lipophilic naproxen.

In order to understand the mechanisms by which the solutions and nanoemulsions had acted as permeation enhancers, we also estimated the underlying J_{max} determinants of SC solubility and SC diffusivity. Ethanol, used as a cosurfactant in these nanoemulsions, has been shown to extract SC lipids and perturb barrier function by improving the permeation of more hydrophilic drugs such as caffeine through skin by partitioning between the vehicle and SC. Peltola et al.[29] also reported enhanced transdermal delivery of the lipophilic steroid oestradiol from microemulsions containing ethanol as a cosurfactant, with which our findings are consistent. Interestingly, ethanol has been reported to act synergistically with

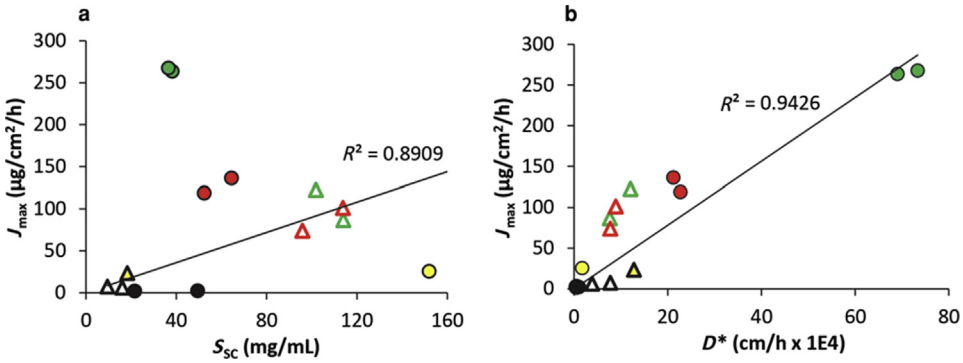

Figure 4. Impact of SC solubility and apparent diffusivity D^* on the maximum flux J_{max} for caffeine and naproxen. (a) J_{max} versus SC. (b) J_{max} versus D^*. Circles, caffeine. Triangles, naproxen. Black symbols, controls (solutions in water and 25% PEG6000/water for caffeine, 25%PEG6000/water, and Volpo-N10/EtOH/water for naproxen). Yellow symbols, 60% EtOH–water. Red symbols, nanoemulsions containing OA. Green symbols, nanoemulsions containing EU. The trend line for naproxen is shown. It is evident from Figure 4a that the J_{max} is more dependent on SC solubility for naproxen, whereas in Figure 4b, J_{max} is better related to D^* for caffeine.

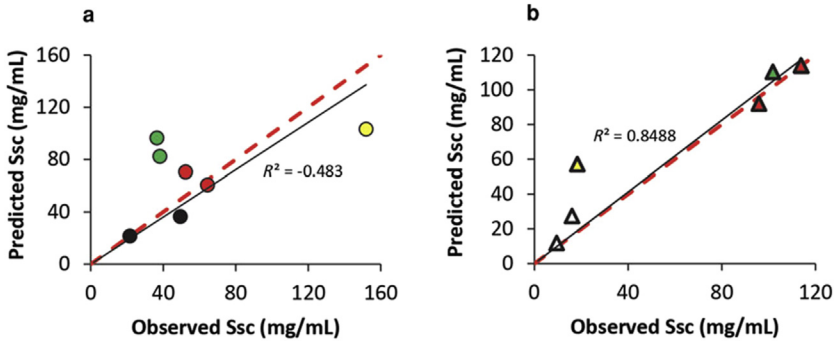

Figure 5. Stratum corneum solubility was predicted from solvent uptake for caffeine (a) and naproxen (b). (Lin's concordance correlation coefficient of caffeine is 0.58 and naproxen is 0.93). Black symbols, control solutions in water and 25% PEG6000/water for caffeine, 25% PEG6000/water, and Volpo-N10/EtOH/water for naproxen). Yellow symbols, 60% EtOH–water. Red symbols, nanoemulsions containing OA. Green symbols, nanoemulsions containing EU.

terpenes to enhance penetration of both hydrophilic and lipophilic drugs.[30,31] The increased ethanol content in the 60% ethanol–water mixture, however, was likely to have a greater effect on the skin, particularly on extraction of skin lipids. This would have a larger impact on the partitioning of the hydrophilic caffeine, compared with the lipophilic naproxen, accounting for the much greater enhancement of S_{SC} seen with caffeine. It should be recognized, however, that at higher levels, ethanol may have a dehydrating effect, resulting in reduced skin permeation.[32]

Table 3 showed that the SC solubility of naproxen was enhanced by the nanoemulsions much more than that for caffeine (range, 96-114 mg/mL compared with 36-64 mg/mL) and that this increased SC solubility was, in turn, associated with an enhanced flux (Fig. 4). In contrast, there was no such clear relationship seen with caffeine (Fig. 4). The points for caffeine are seen as outliers because increased SC solubility is not the driving force behind caffeine flux. On the contrary, the apparent linear relationship between effective diffusivity in the epidermal membrane and J_{max} for caffeine (Fig. 4) suggests that the nanoemulsion formulations may have modified the SC lipids.[33] In contrast, the J_{max} for naproxen appears to not have been increased by alterations in the stratum corenum diffusivity. Consequently, the points for naproxen flux are seen as outliers compared with the apparent linear relationship between flux and diffusivity seen for caffeine. In general, diffusivity will be independent of solute lipophilicity for those vehicles that do not affect the SC.[34,35] However, when the vehicle does affect the SC, there may be varying impacts for different types of solutes. We have found, for instance, an increased diffusivity for the more hydrophilic phenols applied in isopropyl myristate (IPM) relative to more lipophilic phenols. IPM has a high affinity for skin and can disrupt the lipid bilayer regions of the SC intercellular matrix. Its SC diffusivity enhancement was most pronounced for moderately

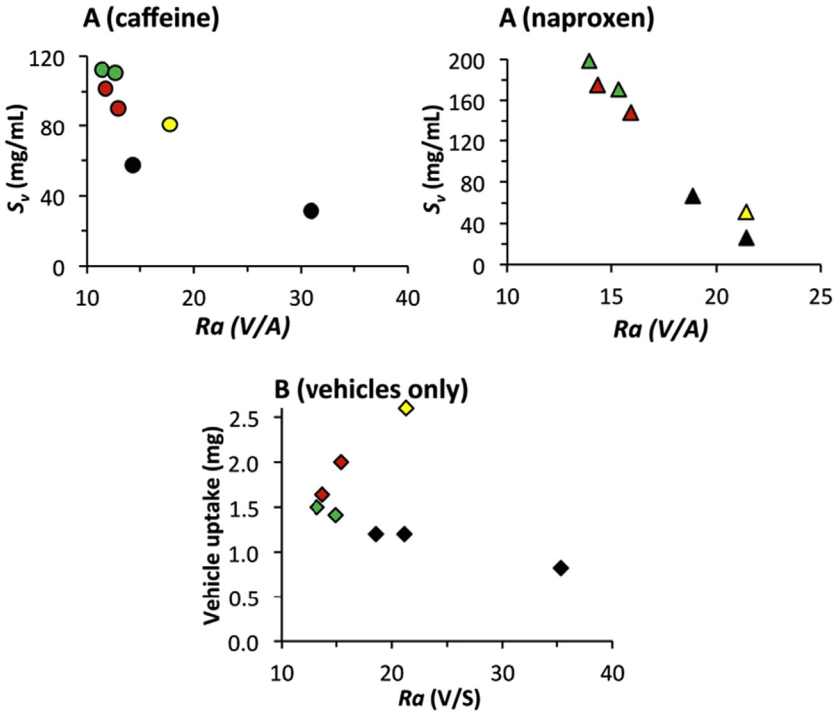

Figure 6. Solute solubility in vehicle and formulation uptake into SC as a function of their estimated HSP distance (Ra). (a) Vehicle solubility (S_V) for caffeine (circles) and naproxen (triangles) versus the HSP distance between the nanoemulsion formulations and controls [Ra (V/A)]. (b) Formulation uptake into the SC (diamonds) versus the HSP distance between the nanoemulsion formulations and the skin [Ra (V/S)]. Black symbols, controls (solutions in water and 25% PEG6000/water for caffeine, 25% PEG6000/water, and Volpo-N10/EtOH/water for naproxen). Yellow symbols, 60% EtOH–water. Green symbols, nanoemulsions containing EU. Red symbols, nanoemulsions containing OA.

hydrophilic compounds such as methyl paraben (log *P* 1.95) and 4-propoxyphenol (log *P* 2.34) and so may be anticipated to also affect caffeine (log *P* −0.07), consistent with the nanoemulsion enhancement of its diffusivity seen in this work. Interestingly, we also observed a reduced diffusivity for the more lipophilic phenols (above log P of about 3.0) from IPM,[35] consistent with our findings with naproxen. Our findings of diffusivity mediated caffeine flux increases here, which were particularly evident in the nanoemulsions containing EU, are consistent with previous work showing increased diffusivity of the polar compound 5-FU with terpene enhancers, including EU.[33] In that work, the terpenes were not considered to act by increasing SC solubility but by disruption of the SC lipids to enhance diffusivity. On the contrary, in other work, OA had only a moderate diffusion-enhancing effect compared with the terpene d-limonene for solutes with a range of lipophilicities,[36] analogous to our findings. Terpenes have also been shown to enhance the permeation of a lipophilic compound, tamoxifen, by enhancing its partitioning into the SC.[37] Williams and Barry[38] observed that in general, partitioning may play a greater role in enhancement of more lipophilic substances. Another contribution to enhanced flux seen with the nanoemulsions could come from an increase in surface area coverage, because of distribution of the drug in the nanoemulsion droplets to aid in the transfer of the active from the formulation to the SC.[16,29]

A key finding in this work is that nanoemulsion-enhanced SC solubility is because of the uptake of solute dissolved in the formulation and this, in turn, depends on the solubilities of the solutes in the formulations and formulation uptake into the SC. We did explore whether the relationships between the solubilities of the active compounds in the various formulations and in the SC after application of the formulations with the HSP distance for the nanoemulsions and actives (Fig. 6). Our conclusion is that the data are consistent with the adage of “like dissolves like,” in that highest solubility in the formulations and uptake of formulations into the SC corresponded to the lowest HSP distance. The findings appear to be consistent with the general principles enunciated by Abbott et al.[39] Further studies using an expanded range of vehicles and actives may allow predictive algorithms to be generated.

Conclusions

The nanoemulsion systems in this study significantly enhanced the human epidermal permeation of caffeine and naproxen. Both a nanoemulsion increase in SC solubility and in diffusivity could be shown as the key mechanisms for this increase with the enhanced solubility arising from solute being carried into the SC with the formulation. Analysis of this data using solubility parameters suggests that “like dissolves like” is the key adage determining solute solubility in the formulations, uptake of the formulations into the SC, and thence the SC solubility.

Acknowledgments

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