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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).