Penetration enhancing effects of selected natural oils utilized in topical dosage forms

Joe M Viljoen *, Amé Cowley, Jan du Preez, Minja Gerber and Jeanetta du Plessis

Centre of Excellence for Pharmaceutical Sciences, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa.

* Corresponding author: Joe M. Viljoen, Ph.D., Department of Pharmaceutics, Faculty of Health Sciences, School of Pharmacy, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa. Email: 11320036@nwu.ac.za; Tel.: +27-18-299-2273; Fax: +27-18-299-2248.

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**Abstract**

**Context:** Various natural products, including oils, have been utilized as penetration enhancers due to their “safety profiles”. These oils contain fatty acids promoting skin permeability through lipid fluidization within the stratum corneum; and might therefore be able to effectively enhance transdermal drug delivery.

**Objective:** We investigated possible penetration enhancing properties of selected oils, utilizing flurbiprofen as marker compound in emulgel formulations. The formulations were compared to a liquid paraffin emulgel and a hydrogel to establish any significant penetration enhancing effects.

**Methods:** Gas chromatographic analysis of the natural oils was performed at ambient temperature to determine the fatty acid composition in each selected natural oil. Franz cell diffusion studies and tape stripping methods were employed to study delivery of the marker into, and through the skin.

**Results:** The following rank order for the emulgel flux-values was obtained: Hydrogel >>> olive oil >> liquid paraffin >> coconut oil > grapeseed oil >> Avocado oil ≥ Crocodile oil >> Emu oil.

**Discussion:** Results suggested that oils containing predominantly mono-unsaturated oleic acid, on average increased the flux of the marker to a larger extent than oils containing an almost even mixture of both mono- and polyunsaturated fatty acids. Oils comprising saturated fatty acids (SFAs) with alkyl chains between C_{12} and C_{14}, increased the marker flux to a higher extent than oils containing C_{16}-C_{18} SFAs. Effects observed for branched fatty acids, however, did not vary significantly from effects for unbranched fatty acids with the same carbon chain length.

**Conclusion:** Natural oils possess penetration enhancing effects.
Introduction

From 2000 to 2012 the worldwide transdermal market doubled its worth to more than US$ 4 billion and is centered on only limited approved drugs\textsuperscript{1}. It is the most widely practiced non-oral systemic drug delivery system. However, transdermal drug delivery is not suited for all drugs and it is not justified for all therapies. Furthermore, the skin's barrier function, which controls the permeation of compounds and their rate, is accomplished entirely by the outermost few micrometers of the skin – the stratum corneum (SC)\textsuperscript{1-9}. It is a compositionally and morphologically distinctive biomembrane that is exceptionally thin, the least permeable of the skin layers, and it is the final stage in epidermal differentiation, forming a shield of compacted keratin-filled corneocytes anchored in a lipophilic matrix\textsuperscript{2,6,10-13}. Given these limitations, especially the lipophilic nature of the SC, the physicochemical properties of compounds are of utmost importance to establish successful transdermal delivery. These properties determine the rate and extent of permeation across the SC via the intercellular lipids, which is considered the main penetration route into the skin\textsuperscript{1,4,13}.

The simplest advances in optimizing transdermal drug delivery entail the manipulation of drug concentration in a vehicle as well as the drug’s SC-vehicle partitioning coefficient. This can be accomplished by using chemicals or penetration enhancers that can reversibly compromise the skin’s barrier function and hence allow the entry of otherwise poorly penetrating compounds into the membrane and through to the systemic circulation\textsuperscript{8,10,14-19}. Compounds reported to render the SC more permeable include alcohols, alkanes, amines, amides, esters, fatty acids, phospholipids, pyrrolidones, sulfides, terpenes, and surfactants – this list is by no means complete\textsuperscript{10,14,16,17,20-24}. However, according to Charoo et al.\textsuperscript{25} numerous compounds that have demonstrated successful penetration enhancing abilities unfortunately possess localized and systemic side effects. Further investigation into possible penetration enhancers that are easy to obtain, effective, safe and cost-effective is therefore desirable.
Various natural products, including oils, have been utilized as penetration enhancers due to their “safety profiles”\textsuperscript{25,26}. It is their unsaponifiable fractions (such as fatty acids, for example oleic acid) that are thought to contribute to their penetration enhancing activities\textsuperscript{22,27-31}. Free fatty acids which are released when natural oils (lipophilic substances) are metabolized within the skin\textsuperscript{32} can be divided into two groups, namely; (i) saturated fatty acids (SFAs) and (ii) unsaturated fatty acids (UFAs). Linoleic and linolenic acids are the most important polyunsaturated fatty acids (PUFAs) and are also called essential fatty acids (EFAs), because the human body lacks the enzymes to manufacture them\textsuperscript{33,34}. SFAs and UFAs have been recognized to effectively enhance transdermal delivery of drugs. They enhance the skin’s permeability by disordering the ordered alkyl chains inside the intercellular spaces, resulting in lipid fluidization within the SC. This modulation of the intercellular lipids of the SC thus increases the permeability of the co-administered compounds or drugs\textsuperscript{8,10,14,16,26,35-40}. The degree of penetration enhancement is affected by (i) the fatty acid chain length, (ii) the presence of double bonds, and (iii) the formulation in which the fatty acid is incorporated\textsuperscript{17}. A general trend found in literature is that UFAs, especially with C-18 alkyl chains (for example oleic acid) are more effective penetration enhancers than SFAs. It is however believed that SFAs with alkyl chain lengths of approximately 10 to 12 carbon atoms, and attached to a polar head group, may also have enhancing abilities\textsuperscript{8,22,35,38}. Chi and co-workers\textsuperscript{41} found a significant increase in the diffusion rate of through rat skin by UFAs, whereas the flurbiprofen permeation increase with SFAs was considered insignificant. Fatty acids also have the advantage of being endogenous components of human skin; and their structural differentiation affects their abilities as penetration enhancers\textsuperscript{42}.

The purpose of the study reported here was to investigate the penetration enhancing characteristics of selected oils of both plant and animal origin on a lipophilic marker (flurbiprofen, log P 4.16)\textsuperscript{43}. Avocado, coconut, grapeseed, olive, crocodile, and emu oils were chosen due to their use in cosmetic formulations. Emulgel formulations consisting of these oils were produced and compared to a liquid paraffin emulgel as well as a hydrogel.
Numerous studies have been conducted on the penetration enhancement characteristics of different fatty acids. Various natural products, such as natural oils, have also been employed as penetration enhancers. However, little is still known about the enhancing effects due to the different fatty acid fractions and combinations in these natural oils. Thus, the findings from this study highlight the promise natural oils, as whole products, hold to enhance drug penetration into the skin.

**Methods**

Flurbiprofen was obtained from DB Fine Chemicals (Johannesburg, South Africa). Liquid paraffin, Span® 60, Tween® 80, propyl and methyl parabens, HPLC grade methanol, HPLC grade ethanol, potassium dihydrogen orthophosphate (KH$_2$PO$_4$), orthophosphoric acid (H$_3$PO$_4$) and sodium hydroxide (NaOH) pearls (used to prepare the phosphate buffered solution) were from Merck Chemicals (Wadeville, South Africa) and Merck Laboratory Supplies (Midrand, South Africa). Polyethylene glycol (PEG) 400 was obtained from Saarchem (Krugersdorp, South Africa) and xanthan gum from Warren Chem Specialties (Johannesburg, South Africa). Deionized HPCL grade water used in this study was prepared with a Milli-Q® (Millipore, Milford, CA, USA) water purification system. Whatman® filter paper, Parafilm® and Dow Corning® vacuum grease were acquired from Separations (Randburg, South Africa).

The avocado, grapeseed and olive oils were obtained from Nautica Organic Trading (Umhlanga, South Africa) and the emu oil from Emuphoria (Potchefstroom, South Africa). Crocodile oil was kindly donated by Croc City Crocodile Farm (Sandton, South Africa); coconut oil by ENCO Fuels (Potchefstroom, South Africa); and the pharmaceutical grade aerosol gas, Solkane® 134a pharma, used as propellant in the manufacturing of the foams, was donated by Solvay Chemicals (Rheinberg, Germany). All other chemicals and solvents used were of analytical grade.
**Fatty acid methyl ester (FAME) analysis**

A GC analysis of the natural oils was performed using a Shimadzu 2010 gas chromatograph (carrier gas flow rate 1.07 ml/min) at ambient temperature to determine the fatty acid composition of each of the selected natural oils. A SGE BP x 70 glass capillary column with an inner diameter of 0.32 mm (film thickness 0.25 μm) and a length of 60 m was used. Nitrogen (N₂) was employed as the carrier gas. The column oven temperature was programmed at 180°C and held for 1 min. It was increased to 225°C at a rate of 10°C/min and held for 4 min, after which the temperature was finally increased at a rate of 10°C/min to 256°C and held for 1 min. All peaks were detected with a flame ionization detector at 310°C.

It was necessary to produce volatile derivatives of the samples in order to analyze the fatty acid composition. Trimethylsulphonium hydroxide (TMSH) was used as transesterification catalyst for the triglycerides in the oils, converting the fatty acids to their corresponding methyl esters. Oil samples were dissolved in 200 μl chloroform to which 200 μl of a 0.2 mol/L solution of TMSH in methanol (50:50) was added. All samples (0.5 μl) were vortexed prior to injection, in duplicate onto the GC, with a split ratio of 1:100 and an inlet port temperature of 230°C.

Quantitative FAME analysis provided percentage values of lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and linolenic acids (C18:3) in each of the natural oils tested.

**Formulation of topical dosage forms**

Six natural oils were selected in order to formulate each into an emulgel; and their penetration enhancing effects were compared to an emulgel containing liquid paraffin as well as a hydrogel that did not contain any oil. An emulgel is defined as a combination of an emulsion and a gel that is used for hydrophobic drug delivery. A hydrogel, on the other hand, consists of an aqueous dispersion medium that is gelled with a suitable hydrophilic gelling agent such as xanthan gum.
The six natural oil emulgel formulations and the liquid paraffin emulgel consisted of 20% oil. All the formulations, however, contained 1% flurbiprofen as a marker in order to distinguish between the penetration enhancing properties of the selected oils. Flurbiprofen has a short elimination half-life (3-4 h) and a rather high log P-value of 4.16, rendering it a highly lipophilic compound. These properties are indicative of a possibly low permeation rate through the skin as the ideal log P-value for topically applied drugs is between 1 and 3. Thus, drug permeation will be the rate limiting step for absorption4,46,47.

Two of the emulgels that presented with the highest flux-values were placed in new foam containers and sealed. A propellant (Solkane® 134a pharma) was forced under pressure into the containers in order to produce the final foam formulation.

Each emulgel thus consisted of 1% flurbiprofen, a natural oil/liquid paraffin (oil phase), Span® 60 and Tween® 80 (emulsifiers and non-ionic surfactants), methyl and propyl parabens (preservatives), ethanol and PEG 400 (polar solvents for flurbiprofen), xanthan gum (polymeric thickener/gelling agent) and Milli-Q® water (solvent).

**Emulgel formulations containing natural oils or liquid paraffin**

Emulgel formulations containing either a selected natural oil or liquid paraffin were prepared by weighing the ingredients into two separate glass containers. Container 1 consisted of 1% flurbiprofen; a mixture (50:50) of ethanol and PEG 400 (6%), which was used to dissolve the flurbiprofen (active phase); and an oil phase. The oil phase containing either a natural oil or liquid paraffin (20%); Span® 60 (3.55%); Tween® 80 (1.35%); methyl paraben (0.40%); and propyl paraben (0.08%), was heated to 80°C. Directly after the oil phase reached the appropriate temperature, it was added to the active phase.

A water phase, consisting of xanthan gum (1%) and 66.62 g Milli-Q® water, was prepared in the second glass container. The water was heated to 80°C and the xanthan gum was slowly added in small quantities in order to dissolve it, while stirring the mixture with a glass rod. The subsequent oil phase was added to the water phase and homogenized at 13 500 rpm until cooled to 40°C. Each formulation was allowed to cool to room temperature; and was stored at 2 - 8°C until the following morning in order to conduct the diffusion studies.
**Hydrogel formulation**

The same preparation method described above was used to manufacture the hydrogel formulation. However, the oil phase was omitted and replaced with Milli-Q® water (20%). In order to increase the viscosity of the hydrogel, a higher xanthan gum concentration (1.5%) was added to the formulation.

**Foam formulation**

Previously prepared emulgels (containing either olive oil or coconut oil) were measured in new, open foam containers and sealed by crimping an aluminum valve seat to the containers. A pharmaceutical grade propellant, Solkane® 134a pharma, was forced under pressure into the sealed containers to produce a foam. In order to ensure that equal amounts of propellant were used for each foam, the emulgel together with the gas, were weighed before and after filling the containers. These formulations were prepared in duplicate.

**Permeability studies**

*Membrane permeation experiments*

Prior to skin diffusion experiments, six-hour membrane diffusion studies were performed in order to determine whether the formulations would release the marker (flurbiprofen). These experiments were conducted in the same manner as the skin diffusion experiments (following section), although polytetrafluoroethylene (PTFE) membranes were used instead of excised human skin. Phosphate buffered solution from the receptor compartments were extracted every hour for six hours.

*Franz cell permeation experiments*

Human Caucasian skin was used for skin permeation studies and was donated by anonymous female donors who had undergone abdominoplastic surgery. Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics Committee of North-West University under reference number NWU-00114-11-A5. Full-thickness skin was removed from the abdominal area of female patients and subsequently frozen at -20°C for no longer than 6 months. At the start of each experiment, the skin was
removed from the freezer and left to thaw. All skin samples were visually examined for any defects such as stretch marks and large hair follicles/holes before cutting it into pieces (approximately 2 cm in width, 4 cm in length and 400 μm in thickness) with a Zimmer® electric dermatome (Zimmer® Ltd, Swindon, Wiltshire, UK). Excised skin samples were prepared by wiping them with paper tissue soaked in Milli-Q® water; then wiping once with paper tissue soaked in ethanol in order to remove any remaining residual subcutaneous fats and lipids. The harvested skin samples were tapped dry with tissue paper and placed on Whatman® filter paper with the SC facing upwards. These samples were covered with aluminum foil, placed in Ziploc® plastic bags and kept frozen at -20°C until used (within 24 h).

Preceding skin permeation studies, the skin samples were defrosted at room temperature, punched into approximately 15-mm-diameter circles with a punch and hammer; and mounted onto static Franz diffusion cells (FCs) that are universally employed for in vitro release studies. In order to minimize biological variability among the specimens, each of the permeation studies was performed using a single source of harvested skin.

The 12-hour, in vitro skin permeation studies were conducted using vertical FCs that consisted of a receptor capacity (~2 ml) and a permeation area for the skin samples (~1.1 cm² in diameter). Each receptor compartment was filled with 2 ml phosphate buffered solution (pH 7.4) and the temperature of the cell system was maintained at 37°C using a water bath. The receptor compartment was filled, ensuring that no air bubbles formed underneath the skin or membrane (which might impair permeation) by tilting each FC. A Teflon-coated magnetic stirring bar was placed in each receptor compartment to continuously mix the phosphate buffered solution. Skin or an artificial membrane was mounted between the two compartments of the diffusion apparatus, the skin with the SC facing upwards in the direction of the donor compartment. A semi-solid emulgel formulation (1 ml) or a foam formulation was applied to each of the donor areas. To maintain an invariant concentration with the foam formulations, fresh foam was periodically added to the donor phase until 1 ml of the formulation (excluding the gas in the foam) was reached as the volume of the foams was too high to incorporate 1 ml once off. All donor compartments were
covered with Parafilm® and secured with a plastic cap to prevent evaporation or leakage. Dow Corning® high vacuum grease was used to seal the FCs in order to avoid leakage; and a horseshoe clamp was used to secure the two compartments. Subsequently, the prepared FCs were placed in a tray on a Variomag® stirrer plate (750 rpm) within a water bath (37°C). A total of 12 FCs were employed during each of the skin and membrane diffusion studies. Ten FCs received either an emulgel or a foam formulation containing the marker, whereas the other two cells received placebo formulations. At pre-determined time intervals (20, 40, 60, 80, 100 and 120 min and thereafter at 2, 4, 6, 8, 10 and 12 h) the entire receptor volume of each compartment was withdrawn and replaced with fresh buffer (37°C), ensuring sink conditions. The time intervals for the membrane release studies were hourly up to 6 h. Samples from the receptor compartments were directly assayed on HPLC without any further processing.

**Tape stripping procedure**

All the skin circles were removed after each of the 12 h permeation studies. The skin circles, with the SC facing upwards, were fixed onto Whatman® filter paper, which was placed on a flat surface. Excess formulation was dabbed from the skin with a clean paper towel and the circular imprints from the donor phase showed the diffusion area. To perform tape stripping, 16 strips of 3M Scotch® Tape were needed. The first strip was discarded as it was considered part of the cleaning procedure. The next 15 strip samples taken from the specific skin section were all placed in a single polytop containing 5 ml phosphate buffered solution. A glistening epidermal layer indicated that the entire SC was removed. The remaining epidermis-dermis diffusion area of each skin sample was cut into small pieces and placed into a polytop containing 5 ml of a 40% methanol in phosphate buffered solution. The polytops with the SC-epidermis and epidermis-dermis samples were vigorously shaken to wet all the strips and kept overnight for 12 h at 4°C. Samples were extracted with 0.45 μm syringe filters and analyzed by HPLC the following day.
Sample analysis

Samples collected from the donor phase of the FCs for all the diffusion studies and tape stripping experiments were analyzed by means of HPLC in order to accurately determine the concentrations of the diffused flurbiprofen within these samples. This HPLC method was developed and validated at North-West University, Potchefstroom Campus, South Africa. An Agilent® 1100 series HPLC system, equipped with an Agilent® 1100 pump, diode array detector, UV detector, autosampler injection mechanism and Chemstation Rev. A.10.03 software for data acquisition and analysis (Agilent Technologies, Palo Alto, CA, USA), were used. A high performance, silica-based, reversed phase C18-2, 5 μm particle size, endcapped, 150 x 4.60 nm column (Venusil XBP Agela Technologies, Newark, DE, USA) was employed. The mobile phase consisted of a mixture of acetonitrile, Milli-Q® water and acetic acid (70:30:1). It was degassed using an ultrasonic bath prior to use. The flow rate was set at 1.0 ml/min and the injection volume to 25 μl. UV detection of flurbiprofen was set at 247 nm with a run time of 8 min. The retention time of flurbiprofen was approximately 3.9 min.

Approximately 10 mg flurbiprofen was accurately weighed and transferred to a 100 ml volumetric flask in order to prepare a standard solution. It was made up to volume with a phosphate buffered solution. From this solution, 5 ml was diluted to 50 ml with the phosphate buffered solution (standard solution). Approximately 2 ml of this standard solution was transferred into an HPLC autosampler vial and injected in duplicate at different volumes in order to establish a concentration range. The values of the obtained peak areas were plotted against time to calculate the concentration of the flurbiprofen in relation to a linear regression line.

Statistical analysis

The cumulative concentration (μg/cm²) of the marker (flurbiprofen) from each formulation that permeated the skin was plotted against time; the slope of the resulting straight line was used to determine the flux. All 10 FCs from each experiment containing the marker were initially
used, but some outlier cells were omitted from the data due to leakage of the cells, abnormal flow, or depletion prior to completion of experiments.

Inferential statistics used in these experiments involved the analysis of variances (ANOVA), as well as a non-parametric, hypothesis testing. For the investigation of significant differences between the flux-values, one-way ANOVA was employed. It was followed by a post-hoc Tukey test that identified formulations which differed from one another. The Kruskall-Wallis test was performed to generate group medians, actual p-values and test statistics. Through this test it was found that the median flux-values would be a more accurate measurement of the true flux-values.49,50.

In addition, a Dunn's multiple group comparison was conducted in order to investigate the accumulation concentration of the marker using tape stripping data obtained from the SC-epidermis and epidermis-dermis. A two-way ANOVA for significant interactions was performed. This was followed by a one-way ANOVA and non-parametric, Kruskall-Wallis test for the SC-epidermis and epidermis-dermis, respectively, followed by a Dunn's multiple group comparison. Inferential statistics were performed at a 5% level of significance where p < 0.05 was indicative of a statistically significant difference49.

Results and discussion

Fatty acid methyl ester (FAME) analysis

FAME analysis of the natural oils revealed high oleic acid (MUFA) concentrations in olive, avocado, emu and crocodile oils (Table 1). Lower oleic acid concentrations were recorded in grapeseed and coconut oils. A high linoleic acid (PUFA) concentration was present in grapeseed oil, whereas the other oils presented significantly lower linoleic acid levels (< 25%). Coconut oil yielded a high lauric acid (SFA) concentration and a medium myristic acid (SFA) concentration. Palmitic acid (SFA) was present at medium levels in crocodile and emu oils, whereas avocado, grapeseed, olive and coconut oils returned palmitic acid levels below 15%. Although stearic acid concentrations were measured in all of the tested natural
oils, these levels were not notably high. These results correlated well with those in the literature\textsuperscript{51,52}.

**Table 1.** GC results of the fatty acid composition (%) of the selected natural oils.

### Permeability studies

**Membrane release experiment**

Membrane release experiments were only conducted in order to establish if the marker was released from the formulations. All of the formulations released detectible amounts of flurbiprofen as all showed some degree of diffusion through the membranes into the receptor compartments during the membrane permeation experiments. The following rank order for the emulgel formulations was established on the basis of the average percentage flurbiprofen diffused and the median flux-values recorded: emu oil (145 µg/cm\textsuperscript{2}.h) ≥ liquid paraffin (137 µg.cm\textsuperscript{2}.h) >> olive oil (110 µg/cm\textsuperscript{2}.h) > crocodile oil (104 µg/cm\textsuperscript{2}.h) = coconut oil (101 µg/cm\textsuperscript{2}.h) ≥ hydrogel (94 µg/cm\textsuperscript{2}.h) >> avocado oil (65 µg/cm\textsuperscript{2}.h) > grapeseed oil (48 µg/cm\textsuperscript{2}.h). Statistical analysis (one-way ANOVA, Tukey post-hoc comparison and non-parametric Kruskal-Wallis test) revealed significant differences between the release rates of the different formulations. However, the more conservative Dunn's, non-parametric, multiple comparison test revealed smaller differences among these formulations; and it was established that no significant differences were observed between the formulations containing avocado oil, grapeseed oil or the hydrogel. Formulations containing oils high in oleic acid (MUFA) concentrations, therefore, released more flurbiprofen than those containing oils with relative high concentrations of linoleic acid (PUFA, i.e. branched-chain fatty acids). Moreover, formulations containing oils high in lauric acid (SFA) released more flurbiprofen than those containing oils with an almost equal mixture of MUFAs and PUFAs.

**Transdermal delivery experiments**

The lipophilic nature of flurbiprofen as well as its chemical structure affects its solubility and partitioning characteristics, thus ensuring good solubility and easy partitioning between the hydrophilic and lipophilic regions of the SC, resulting in higher concentrations within this
Furthermore, the significantly high diffusion rate \((p < 0.05)\) into the skin and accumulation in the SC displayed with the hydrogel (Figure 1) could have been caused by these characteristics as well as flurbiprofen's poor solubility within the hydrophilic formulation, causing it to partition readily from the formulation into the skin\(^{53,54}\). Due to the aqueous regions within the skin, however, flurbiprofen only slightly partitioned out and into the epidermis-dermis\(^{19,53-55}\). A propensity for flurbiprofen to diffuse from certain formulations into the viable epidermis could have been the reason why lower concentrations were obtained in the SC\(^{4,55}\).

**Figure 1.** *Box-plot representation of the flurbiprofen concentrations measured in the stratum corneum-epidermis after it was released from the different formulations.*

The average concentrations (dashed red line) and median concentrations (solid line) can also be seen.

Hydration of the SC due to additional water; in this case from the hydrogel formulation, also alters permeant solubility and thereby modifies partitioning from the vehicle into the membrane. Additionally, increased skin hydration may swell and open the SC structure, i.e. the packed horny layer, leading to an increase in penetration. Moreover, hydration can be improved by occlusion with plastic films; paraffins; oils; waxes as components of ointments; water-in-oil emulsions that prevent transepidermal water loss (TEWL); and oil-in-water emulsions that donate water. Of these, occlusive films consisting of plastic, fat or an oily vehicle have the most insightful effect on hydration due to the prevention of TEWL, and thus also on the penetration rate. Fatty acids contained in most of the oils tested in this study, are lipophilic and can therefore also hydrate the lipid structures within the SC by means of a swelling process\(^{5,16,21,56-60}\).

The following rank order for the emulgel formulations was determined on the basis of the average flurbiprofen concentration measured in the SC-epidermis following skin diffusion experiments (Figure 1): olive oil foam \((21 \, \mu g/cm^2)\) = liquid paraffin \((21 \, \mu g/cm^2)\) > olive oil \((18 \, \mu g/cm^2)\) = grapeseed oil \((18 \, \mu g/cm^2)\) ≥ hydrogel \((17 \, \mu g/cm^2)\) > crocodile oil \((15 \, \mu g/cm^2)\)
coconut oil (7 µg/cm²) > avocado oil (3 µg/cm²) > coconut oil foam (2 µg/cm²) ≥ emu oil (1 µg/cm²).

Higher flurbiprofen accumulation in the SC was obtained with formulations comprising oils high in UFAs (> 70%). Formulations containing either olive oil or crocodile oil are rich in oleic acid (C18:1), a MUFA that has been classified as having good penetration enhancing effects. The grapeseed oil emulgel, conversely, was rich in linoleic acid (C18:2) which is a PUFA. Linoleic acid consists of two double bonds in its carbon chain which causes conformational kinks. The more kinked-shaped cis-double bonds present, the more difficult it becomes for fatty acids to insert themselves within the lipid bilayers of the SC. Thus as a result, the lag time is increased; however once they are packed in the skin, the channels in the SC are enlarged, which in turn causes higher skin flux of the drug. This increase in the flurbiprofen flux resulted in the accumulation of flurbiprofen in the SC and caused a reservoir effect.

Lower flurbiprofen accumulation in the SC was obtained with formulations consisting of avocado, coconut and emu oils. Avocado and emu oils contain higher concentrations SFAs, especially fatty acids with carbon chain lengths longer than C-12 (e.g. palmitic and stearic acids). The longer chain SFAs have a higher affinity toward the lipids within the SC as a result of the lipophilic nature and may, therefore, have delayed permeation of flurbiprofen into the skin due to hydrophobic interactions. Conversely, coconut oil consists of higher concentrations short-chain SFAs (e.g. lauric acid). These fatty acids have inadequate lipophilic properties to actively permeate the SC, thus, rendering poor penetration.

Overall, lower flurbiprofen concentrations obtained in the epidermis-dermis might have been due to its lower solubility within the aqueous environment of the epidermis-dermis, and it thus rather residing within the SC. Pellet et al. stated that the flux of a constituent across a membrane from a given formulation, whether or not penetration enhancers are included in the formulation, is restricted by its saturated solubility within the vehicle. If the formulation contains a saturated concentration of the permeant, its concentration in the outer layers of the SC will also be saturated if the permeant is soluble in these layers. Thus, the chemical
potential gradient will be enhanced: the driving force for diffusion across the SC is therefore elevated in proportion, in this manner allowing penetration into the epidermis-dermis. The flurbiprofen formulations tested in this study were however not saturated, thereby restricting diffusion into the epidermis-dermis. Protein binding of the hydroxyl groups of flurbiprofen within the SC might also have retarded penetration into the epidermis.

A rank order for the emulgel formulations was established on the basis of differences in the average flurbiprofen concentrations measured in the epidermis-dermis (Figure 2): emu oil (16 µg/cm²) >>> hydrogel (5 µg/cm²) = liquid paraffin (5 µg.cm²) ≥ avocado oil (4 µg/cm²) ≥ coconut oil (3 µg/cm²) = grapeseed oil (3 µg/cm²) ≥ olive oil (2 µg/cm²) = olive oil foam (2 µg/cm²) ≥ crocodile oil (1 µg/cm²) = coconut oil foam (1 µg/cm²).

Figure 2. Box-plot representation of the flurbiprofen concentrations measured in the epidermis-dermis after it was released from the different formulations. The average concentrations (dashed line) and median concentrations (solid line) can also be seen.

A significantly high comparative flurbiprofen concentration (p < 0.05) attained in the epidermis-dermis with the emu oil emulgel could have been due to obvious scaling of the epidermis as a result of the relatively high concentrations oleic (C18:1) and linoleic (C18:2) acids present within this oil. Emu oil is relatively high in linolenic acid (C18:3), a PUFA that might have trapped the marker within the epidermis-dermis due to the degeneration of the skin appendages. Scaling of the epidermis-dermis might have caused abnormal epidermal proliferation and differentiation (thickening). Fatty acids containing unsaturated alkyl-chains of C-18 (UFAs) appear to cause optimum scaling and their bent cis-conformation, as stated before, is believed to disrupt the intercellular lipid packing of the SC. It is therefore plausible that this modification causes accumulation of flurbiprofen in the epidermis-dermis region, creating a reservoir effect. Oleic acid is thought to cause hyperplasia of the SC and epidermis-dermis as a result of an inflammatory response with resulting epidermal proliferation. However, high oleic acid concentrations that remained in the SC after it was
released from the emulgels containing avocado, grapeseed, olive or crocodile oils might have caused an increase in TEWL and morphological changes in this region. These changes might have resulted in small amounts of flurbiprofen diffusing into the epidermis-dermis region. The formulations containing higher oleic acid concentrations caused more morphological changes in the SC and thus triggered more flurbiprofen to accumulate in the SC\textsuperscript{61,67}. High oleic acid levels also increased the partition coefficient of flurbiprofen rendering the olive oil a powerful enhancer. The diffusion coefficient of a drug is increased as the penetration enhancer forms micro-cavities within the lipid bilayers due to fluidisation, thus, increasing the free volume fraction.

Avocado and coconut oils contain higher SFA concentrations compared to the other oils. Formulations containing these oils did not exhibit any significant accumulation of flurbiprofen in the epidermis-dermis. These findings might be accredited to the difference in lipid solubility and structure between SFAs and UFAs. Lauric- (C12:0), myristic- (C14:0) and palmitic- (C16:0) SFAs have melting points in the range of 31 - 59°C, which are significantly higher than UFAs (-50 - 4°C). Subsequently, it is expected that these SFAs will have lower solubility. The avocado and coconut oils were semisolids at the temperatures at which the experiments were conducted, confirming the statement. Lauric-, myristic- and palmitic acids are of linear shape, which additionally lessened their capability to disrupt the lipid packing of the SC and to insert themselves into the lipid bilayers, resulting in little or no effect(s)\textsuperscript{29,41}.

Significantly lower flurbiprofen concentrations (p < 0.05) obtained by the hydrogel and liquid paraffin formulations in the epidermis-dermis compared to the SC were observed. As previously stated, these formulations hydrated the SC, which generally facilitates permeation of hydrophilic compounds through the SC and constrains lipophilic compounds, such as flurbiprofen, from diffusing to the epidermis-dermis. Hydration of the SC, however, also alters the intercellular lipid domain of the skin causing structural defects (fluidization) of the lipid pathway that leads to the formation of small mobile free volumes. The resistance of this newly formed liquid lipid phase decreased accordingly; enhancing partitioning of flurbiprofen into the epidermis-dermis and thus not completely preventing diffusion\textsuperscript{5,16,61,62,68}.  

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Comparison of the median flurbiprofen flux-values (Figure 3) through excised human skin obtained after diffusion studies (12 h) produced the following rank order for the emulgel formulations: hydrogel (24 µg/cm².h) >>> olive oil (18 µg/cm².h) > liquid paraffin (16 µg.cm².h) >> coconut oil (13 µg/cm².h) ≥ grapeseed oil (12 µg/cm².h) >> avocado oil (8 µg/cm².h) ≥ crocodile oil (7 µg/cm².h) ≥ olive oil foam (6 µg/cm².h) > emu oil (4 µg/cm².h) = coconut oil foam (4 µg/cm².h).

**Figure 3.** *Box-plot representation of the flux values for the different formulations. The average flux values (dashed line) and median flux values (solid line) can also be seen.*

The hydrogel formulation depicted the highest flux-value which, as previously explained, was probably due to the high water content of this formulation causing hydration of the skin and subsequent swelling of the SC with an increase in flurbiprofen permeation5,7,16,58-60,64. The liquid paraffin formulation, on the other hand, caused skin occlusion that resulted in swelling of the corneocytes, which in turn increased the water content within the intercellular lipid region of the SC, preventing TEWL with a subsequent increased flurbiprofen flux31,38,69. Even though liquid paraffin preparations have been used as penetration enhancers before, it has been shown that it can cause early depletion of the drug from a formulation70. El Magraby et al.71 furthermore stated that care should be taken when hydrating the skin as an overhydrated skin could produce too much swelling of the corneocytes and could thus negatively affect permeation of an active ingredient.

The olive oil emulgel depicted a significantly higher (p < 0.05) flurbiprofen flux compared to the other emulgels. As quantified, olive oil contains a high oleic acid (C18:1) (MUFA) concentration; and significantly lower SFA and PUFA concentrations. This was in agreement with results obtained by Chi et al.41 who stated that UFAs which increased the partition coefficient of flurbiprofen were powerful enhancers. Oleic acid has been shown to increase permeability due to its bulky “kink” conformation that fluidizes the extra-cellular lipid area of the SC. The diffusion coefficient of a drug is increased as the penetration enhancer forms
micro-cavities within the lipid bilayers, thus, increasing the free volume fraction. Oleic acid, predominantly present in a high concentration, pool within the extra-cellular lipid area to create permeable ‘pores’ that provide less resistance and an increased TEWL\textsuperscript{36,41,61,72}. Interestingly, UFAs affect the activity of enzymes regulating the desquamation of the SC, and as a result deterioration of the barrier function around the sebaceous glands occur\textsuperscript{66}.

High SFA levels, especially lauric acid (C12:0) and myristic acid (C14:0), present in the coconut oil emulgel were responsible for the increased flurbiprofen flux. However, even though lauric acid has been previously described as an effective skin permeation enhancer for several compounds, in this study only a moderate increase in the flurbiprofen flux was observed. A suggested mechanism of drug permeation enhancement by lauric acid is complex formation between the drug and the fatty acid; followed by dissociation of this complex into each component in the interface between the SC and viable epidermis. The moderate enhancing effect witnessed in our study could be explained by the absence of an interaction between the SFA molecules and flurbiprofen as flurbiprofen and the SFAs are negatively charged at pH 7.4; and they therefore, probably do not form any complex\textsuperscript{42,63,73-75}.

Another suggestion for increased permeability was that an optimal equilibrium may exist between the partition coefficient and the affinity of the hydrophobic groups of the SFAs for the skin. However, as stated previously, SFAs of linear shape and low solubility due to their higher melting points are less capable of disrupting the lipid bilayer of the SC and inserting themselves into the bilayers compared to UFAs\textsuperscript{41,63}. Therefore, only moderate penetration enhancement could be achieved with the coconut oil emulgel.

Grapeseed oil is rich in PUFAs, especially linoleic acid (C18:2) and its oleic acid content is also satisfactory, but its SFA content can be considered as significantly low. This emulgel formulation depicted a flurbiprofen flux that was comparable to the flux-value obtained from the coconut oil emulgel. Even though the oleic acid content of the grapeseed oil emulgel could have increased diffusion of flurbiprofen through the skin, it was probably the high linoleic acid content that hindered permeation enhancement. The more kinked shape a fatty acid becomes due to increased \textit{cis}-double bonds, the more difficult it becomes for the fatty
acid to insert itself into the lipid bilayers, which results in a longer lag time. Nonetheless, once they are packed in the skin, wider channels are formed which may then result in higher flux-values\textsuperscript{41,42}.

The flux-value obtained with the avocado oil emulgel was 2.25 times lower than that obtained with the olive oil emulgel. Avocado oil is rich in oleic acid (MUFA), which as discussed, is a known penetration enhancer. However, it also contains significant palmitic- (C16:0) (SFA) and linoleic acid (PUFA) concentrations. Palmitic acid has an exceptionally high melting point (± 63°C) which negatively affects the solubility of avocado oil and this SFA might not have been dissolved properly at the temperature the study was conducted at\textsuperscript{29,41,69}. Thus, reduced disruption of the SC lipid bilayers occurred and the lag time was prolonged. In addition, its chain length is 16 carbons long and it has been found that an increase in chain length above C\textsubscript{14} does not always result in significant penetration enhancement\textsuperscript{8,63,75}.

Although the more kinked shape linoleic acid has a significantly lower melting point (-6.5°C) that would have had a positive effect on the solubility of the avocado oil, it would also have extended the lag time due to its kinked shape as well as its two double bonds in the carbon chain as explained earlier\textsuperscript{29,41,42}.

Increased flurbiprofen flux-values were expected for the emu and crocodile oil emulgels as these oils are from animal origin and show close similarities to human butter fat\textsuperscript{8,76}. However, the flux-values of these emulgel formulations were respectively 4.5 and 2.6 times lower than the olive oil emulgel flux-value. Compared to the other oils, the crocodile and emu oils both contain significantly higher levels of SFAs (palmitic and stearic acids) and PUFAs (linoleic- and linolenic acids). As previously described, these SFAs have longer carbon chains (> C\textsubscript{14}) and higher melting points (62.9 and 69.6°C, respectively) that decrease the solubility of the oil and limit disruption of the SC lipid bilayers, thus prolonging the lag times\textsuperscript{8,33,41,45,63,69,76}. It is believed that branched versions of stearic acid could upset the packing of the lipids in the SC to a more significant extent\textsuperscript{77}. The emu oil emulgel contains the highest concentration stearic acid and therefore depicted the lowest flux-value. The linoleic and linolenic acids consist of two and three double bonds, respectively, in their
carbon chain which cause conformational kinks. Again, the more kinked-shaped cis-double bonds present, the more difficult it becomes for fatty acids to insert themselves within the lipid bilayers. Thus, the lag times are increased, but once inserted in the skin; the channels in the SC are enlarged, which in turn may eventually cause higher drug flux through the skin\textsuperscript{33,41,61,62}.

**Foam formulations**

The only difference between the two foam formulations and the emulgel formulations was that an aerosol gas (Solkane\textsuperscript{®} 134a pharma) was used as propellant during the production phase in order to create foams from the olive oil and coconut oil emulgels. This trapped air reduced optimal contact with the available skin surface area until the foams collapsed. The air trapped in each foam could also have caused a smaller amount of these formulations present within the donor phase of the FCs during permeation testing. Thus, less flurbiprofen per volume applied compared to the other emulgels, would be available for testing. Despite the aforementioned reasons for low measurements obtained, a relatively high flurbiprofen concentration was measured in the SC-epidermis (Figure 1) which was released from the olive oil foam formulation. As stated earlier, olive oil is rich in oleic acid and this fatty acid has been shown to significantly increase the partition coefficient of flurbiprofen and thus enhance permeation\textsuperscript{41,62,75}. However, significantly low flurbiprofen levels (p < 0.05) were obtained in the epidermis-dermis (Figure 2) as well as significantly low flux-values (p < 0.05) (Figure 3) were measured for the olive oil foam formulation. This was probably due to the reservoir effect that occurred in the SC-epidermis only after the foam finally collapsed. The time it took the foam to collapse and occlude the skin probably delayed the permeation process of flurbiprofen.

Contrary to results obtained with the olive oil foam, the coconut oil foam formulation depicted significantly poor permeability (p < 0.05) of flurbiprofen into the SC-epidermis and epidermis-dermis. This formulation also portrayed a significantly low flux-value. Again, the trapped air reduced optimal contact with the accessible skin and less flurbiprofen were available per volume applied, compared to the other emulgels. However, no significant difference
(p < 0.05) could be established between the flux-values of the two foam formulations. The relatively low measurements obtained in our study could again be explained by the absence of an interaction between the SFA molecules in the coconut oil and flurbiprofen, with no subsequent complex formation\(^{42,63,73-75}\), as well as the linear shape and low solubility of the SFAs that hindered disruption of the SC\(^{41,63}\).

**Conclusion**

All of the selected natural oils demonstrated penetration enhancing properties. These oils are relatively inexpensive, said to be non-irritating and spread well over the skin. During the membrane release studies it was found that formulations containing oils high in oleic acid (MUFA) released more flurbiprofen. Furthermore, formulations containing oils rich in SFAs, specifically palmitic acid, also depicted higher flurbiprofen concentrations released from the formulations. Oils high in PUFAs did however not release flurbiprofen to the same extent. As predicted from the physiochemical properties of flurbiprofen, the lipophilic drug preferred to reside within the lipophilic SC rendering higher concentrations in this region and lower concentrations in the epidermis-dermis. Comparison of the membrane and skin diffusion data led to the belief that the skin itself played a major role in the permeation of flurbiprofen through the skin. It was also evident that hydration due to certain formulation characteristics as well as occlusion of the skin, played significant roles in the increased diffusion rate of flurbiprofen into the skin and accumulation in the SC. However, a propensity for flurbiprofen to diffuse from certain formulations into the viable epidermis could have been the reason why lower concentrations were obtained in the SC. The hydrogel and liquid paraffin formulations, for example, depicted significantly lower flurbiprofen concentrations in the epidermis-dermis and higher flux-values. The hydrogel caused hydration of the skin with subsequent swelling of the SC which increased flurbiprofen permeation. Conversely, the liquid paraffin formulation caused skin occlusion that resulted in swelling of the corneocytes, which in turn increased the water content within the intercellular lipid region of the SC, preventing TEWL with a subsequent increased flurbiprofen flux. However, one needs to bear in mind that
liquid paraffin preparations have been shown to cause early depletion of a drug from a formulation. A general trend observed for formulations that contained the selected oils was that the formulations comprising oils high in UFAs (> 70%) depicted higher flurbiprofen accumulation in the SC.

The emu oil emulgel contains the highest concentration stearic acid and thus portrayed the lowest flux-value due to entrapment in the epidermis-dermis. Oils containing predominantly MUFAs (i.e. oleic acid), on average increased the partition coefficient (and thus the flux) of the marker more significantly than oils containing an almost even mixture of both MUFAs and PUFAs, rendering the olive oil a powerful enhancer. On the other hand, oils comprising SFAs with alkyl chains between C12 and C14, increased the marker flux to a higher extent than oils containing C16-C18 SFAs. Effects observed for branched fatty acids, however, did not vary significantly from effects for unbranched fatty acids with the same carbon chain length.

Although a study of Deng et al.78 showed an increase in the rate at which a drug was delivered from a foam formulation, compared to a cream, solution and ointment formulation; we could not conclude any advantage in using these oils as penetration enhancers in foam formulations. Choosing a formulation where the drug is less soluble than in the SC, will enhance partitioning of the drug into the SC. For example, choosing an aqueous gel for a lipophilic drug; and a lipophilic oil for a water-soluble drug, will create a high driving force for diffusion into the SC and accumulation within this region.

In future it would be important to explore the stability of these oils in topical formulations as UFAs in natural oils might be subjected to peroxidation when exposed to air/light, rendering them more unstable than SFAs due to the double bonds within their carbon structures33,79. We would furthermore want to investigate the penetration enhancing effect when a co-solvent such as propylene glycol is included into the formulas. The rational being that for oleic acid to reach the polar surface of the lipid layers within the SC in larger amounts, it may require a co-solvent that could alter the polarity of the aqueous region and increase the solubility of lipophilic drugs. The enhancing effect of propylene glycol together with the
inclusion of certain fatty acids has shown to have a synergistic effect on permeation across the skin\textsuperscript{36,42,79} and therefore needs to be explored. Also important are the enhancing effects these oils might have when formulated in different topical dosage forms (i.e. creams, ointments, etc.) as well as when a different marker is utilized. The inclusion of a hydrophilic drug might prove interesting due to reports of an inverse relationship between a drug’s lipophilicity and the enhancement effects of a series of fatty acids\textsuperscript{79}. Lastly, other natural oils high in oleic acid include canola, mid-oleic sunflower, peanut, pistachio and almond oils\textsuperscript{80}. Investigation of the penetration enhancement properties of these oils could in future also be worthwhile.

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**Declaration of interest**

The authors report no declarations of interest.

**References**


Tables

Table 1. GC results of the fatty acid composition (%) of the selected natural oils.

<table>
<thead>
<tr>
<th>SFA†</th>
<th>Oil or Fat</th>
<th>Avocado</th>
<th>Coconut</th>
<th>Grapeseed</th>
<th>Olive</th>
<th>Crocodile</th>
<th>Emu</th>
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<td>21</td>
<td></td>
<td>1</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
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<td>Palmitic</td>
<td>16:0</td>
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<td>9</td>
<td>6</td>
<td>12</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Stearic</td>
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<td>3</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Palmitoleic</td>
<td>16:1</td>
<td>6</td>
<td></td>
<td>1</td>
<td>6</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Oleic</td>
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<td>8</td>
<td>27</td>
<td>76</td>
<td>40</td>
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<tr>
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<td>24</td>
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<tr>
<td>Arachidonic</td>
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</table>

* Carbon chain length and number of double bonds

† Saturated fatty acids

‡ Monounsaturated fatty acids

§ Polyunsaturated fatty acids
Figure 1. Box-plot representation of the flurbiprofen concentrations measured in the stratum corneum-epidermis after it was released from the different formulations. The average concentrations (dashed line) and median concentrations (solid line) can also be seen.
Figure 2. Box-plot representation of the flurbiprofen concentrations measured in the epidermis-dermis after it was released from the different formulations. The average concentrations (dashed line) and median concentrations (solid line) can also be seen.
Figure 3. Box-plot representation of the flux-values for the different formulations. The average flux-values (dashed line) and median flux-values (solid line) can also be seen.