ESSENTIAL FATTY ACIDS AS TRANSDERMAL PENETRATION ENHANCERS

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Abstract

The aim of this study was to investigate the effect of different penetration enhancers, containing essential fatty acids (EFAs), on the transdermal delivery of flurbiprofen. Evening primrose oil (EPO), vitamin F and Pheroid™ technology all contain fatty acids and were compared using a cream-based formulation. This selection was to ascertain whether EFAs solely, or EFAs in a Pheroid™ delivery system, would have a significant increase in the transdermal delivery of a compound. Membrane release studies were performed and the results indicated the following rank order for flurbiprofen release from the different formulations: vitamin F >> control > EPO >> Pheroid™. Topical skin delivery results indicated that flurbiprofen was present in the stratum corneum-epidermis and the epidermis-dermis. The average percentage flurbiprofen diffused to the receptor phase (representing human blood) indicated that the EPO formulation showed the highest average percentage diffused. The Pheroid™ formulation delivered the lowest concentration with a statistical significant difference (p < 0.05) compared to the control formulation (containing 1% flurbiprofen and no penetration enhancers). The control formulation presented the highest average flux, with the EPO formulation following the closest. It could thus be concluded that EPO is the most favourable chemical penetration enhancer when used in this formulation.

Keywords: Fatty acid, Transdermal, Flurbiprofen, Formulation, Vitamin F, Evening primrose oil, Pheroid™
1. INTRODUCTION

Formulating a product to deliver the active pharmaceutical ingredient (API) to and promote the diffusion of the API through the skin presents many difficulties. Penetration enhancers were chosen to minimise these difficulties. Essential fatty acids (EFAs) have a wide range of attributes and are nontoxic, making them optimal penetration enhancers. Even on their own, EFAs can be used in the treatment of many skin conditions.1, 2

Generally, the accepted range of the octanol-water partition coefficient (log P) for optimal skin permeation is between 1 and 3 and the aqueous solubility should be more than 1 mg/ml. The optimal molecular weight of an API to diffuse through the skin is less than 500 g/mol.3, 4, 5, 6 Flurbiprofen, used in this study only as a reference, is a highly lipophilic compound (log P = 4.24), with poor aqueous solubility (0.057 mg/ml in water) and has a molecular weight of 244.3 g/mol.7, 8 These physicochemical characteristics are an indication that the API could diffuse through the skin, but with some difficulty due to the lipophilicity, if applied onto the skin.

The aim of this study was to investigate the penetration enhancement abilities of selected products containing EFAs. Evening primrose oil (EPO), vitamin F and Pheroid™ technology all contain fatty acids and were compared to one another as well as to a control using cream-based formulations. These four formulations were chosen to determine whether the EFAs in a Pheroid™ delivery system, or exclusively, will have an effect on the transdermal delivery of a pharmaceutical compound (flurbiprofen used as reference compound). The control formulation contained no EFAs but only flurbiprofen in the cream.

EFAs were chosen for this study since they are natural products that promote the delivery of both lipophilic and hydrophilic compounds.9 Fatty acids are chemical penetration enhancers that are considered to be safe for transdermal use; and have received much attention during the past two decades. 10, 11, 12 Fatty acids enhance permeation through entering the lipid bilayers of the skin and disrupting their ordered domains. They can improve the partitioning of an API into the stratum corneum (outmost layer of the skin) and can form lipophilic complexes with compounds.5 Although fatty acids can be used to enhance the permeation of both lipophilic and hydrophilic APIs; the flux of polar APIs is improved to a larger degree.13 The most effective skin penetration effects were found from saturated fatty acids (SFAs) with C10-C12 chain lengths (e.g. lauric and capric acids). Unsaturated fatty acids (UFAs) with a C18 chain length (e.g. oleic and linoleic acids), however, also increase penetration enhancing effects.14 Chi et al.15 and Viljoen et al.16 both stated that UFAs have more powerful skin permeation enhancing effects, with linolenic acid as the most potent, followed by oleic acid, palmitoleic acid, linoleic acid and arachidonic acid.
EPO consists of approximately 98.00% triacylglycerols (triglycerides), 0.05% phospholipids, and 1.00-2.00% unsaponifiable matter (sterols and tocopherols are of some importance). The key contents of EPO are linoleic acid (70-80%), γ-linolenic acid (GLA: 8-12%), and oleic acid (6-11%) \(^\text{17, 18, 2}\). These fatty acids are found naturally in the skin and are therefore considered as nontoxic \(^\text{10, 11, 12}\).

Older literature often referred to EFAs as vitamin F, since vitamin F mainly contain linoleic- and linolenic acid, the essential fatty acids \(^\text{19}\). These EFAs are unsaturated (contain one or more double bonds in the hydrocarbon chain) and can only be acquired through diet \(^\text{20, 2}\). Vitamin F consists mainly of linoleic (35.5%), linolenic (30.5%), and oleic acid (21.4%) \(^\text{21}\). Linoleic- and linolenic acid are polyunsaturated fatty acids (PUFAs); and are known to enhance the delivery of a variety of compounds \(^\text{11, 12, 9}\).

Pheroid™ technology is a patented drug delivery system consisting of PUFAs, which include omega-3 and omega-6 fatty acids, but it excludes arachidonic acid. The fatty acids used to produce Pheroid™ are emulsified in nitrous oxide saturated water and dispersed in a dispersion medium. However, this technology also contains a dispersed gas phase (the nitrous oxide); giving it the edge as it does have three phases, namely a water phase, an oil phase, and a gas phase. The gas phase contributes to the stability and the self-assembly process of the Pheroid™ \(^\text{22, 23}\). Pheroid™ technology consists mainly of EFAs and has shown no immune responses in humans. Pheroid™ has, furthermore, been shown to enhance bio-availability and cause no cytotoxicity, therefore making it an optimal choice as drug delivery vehicle \(^\text{22, 23}\).

2. MATERIALS AND METHODS

2.1 MATERIALS

Flurbiprofen was obtained from DB fine chemicals®, South Africa. Evening primrose oil (Windrose, South Africa), Pheroid™ and vitamin F (North-West University, Potchefstroom Campus, South Africa) were used as penetration enhancers. Other ingredients used in the formulation of the semi-solid products (liquid paraffin, Tween® 80, and cetyl alcohol) were obtained from Merck® Laboratory Supplies (Midrand, South Africa). Span® 60 was obtained from Fluka® Analytical (Germany). All other ingredients used were of analytical grade.

2.2 METHODS

2.2.1. Formulation of semi-solid product

A cream-based formulation was used for the diffusion studies. The ingredients for this formulation were as follow: flurbiprofen (1.0%), cetyl alcohol (10.0%), Tween® 80 (1.5%), Span® 60 (1.5%), liquid paraffin (12.0%), methanol (6.0%) to dissolve flurbiprofen and the penetration
enhancer (5.0%). HPLC (high performance liquid chromatography) grade water was added to volume.

The cetyl alcohol, Span®, Tween® and liquid paraffin were placed in a beaker and heated to 70°C (the oil phase). Water was heated to 70°C in a separate beaker. The flurbiprofen was dissolved in methanol (6%) and added to the oil phase. Thereafter, the water phase was quickly added to the oil phase and continuously mixed with a homogeniser at 500 rpm until the cream cooled down sufficiently to reach a temperature of 45°C. One of the penetration enhancers was added, whilst continuously stirring the cream, until it reached room temperature (25 ± 0.5°C).

2.2. High performance liquid chromatography (HPLC) analysis

Concentration assays were conducted using an Agilent® isocratic system (Agilent Technologies, Palo Alto, CA), Luna C18-2 column, 150 x 4.6 mm, 5 µm, (Phenomenex, Torrance, CA). The flow rate was set at 1 ml/min and the detection wavelength at 247 nm. The runtime was set at 6 min and the injection volume at 50 µl. The mobile phase consisted of Milli-Q® water (300 ml), acetonitrile (700 ml) and glacial acetic acid (10 ml).

2.2.3. Solubility and distribution coefficient determination of flurbiprofen

Flurbiprofen solubility was determined in water and phosphate buffer solution (PBS; pH 5). An excess amount of flurbiprofen was placed in a polytop containing 5 ml solvent. The contents were continuously stirred and retained at 32°C for a period of 24 h. The solution was filtered through a 0.45 µm filter and analysed by means of HPLC. The experiment was performed in triplicate.

The experimental octanol-buffer distribution coefficient (log D) was determined by pre-saturating equal volumes n-octanol and PBS (pH 5) for 24 h. After 24 h of pre-saturation, the phases were separated. Flurbiprofen (3 mg) was dissolved in pre-saturated n-octanol (20 ml). The flurbiprofen pre-saturated n-octanol (3 ml) was placed in a test tube and pre-saturated PBS (3 ml at pH 5) was added. The test tube was shaken continuously at 32°C for 24 h after which the mixture was centrifuged for 10 min. Finally, the two phases in the test tube were separated using a pipette and the PBS (pH 5) was injected into the HPLC, whereas the n-octanol phase was diluted with methanol (1:4) before injection into the HPLC. This dilution was done to avoid band-broadening caused by pure octanol. The log D was determined in triplicate.
2.2.4. Franz cell diffusion studies

2.2.4.1. Skin preparation

Caucasian human skin donated by anonymous female donors who underwent abdominoplasty surgery was used during skin permeation studies. Informed consent was obtained from all donors. Ethical approval for the procurement and preparation of the skin was provided by the Research Ethics committee of the North-West University under the reference number NWU-00114-11-A5. Full-thickness skin was removed from the abdomen of these female patients and subsequently frozen at -20°C for no longer than 6 months. Prior to the diffusion studies, the full-thickness skin was thawed and visually examined for stretch marks and/or large hair follicles or other defects. The skin was lightly wiped with tissue paper soaked in ethanol to remove excess blood and subcutaneous fat. Thereafter the skin was cut into pieces of approximately 2 cm in width, 4 cm in length and 400 µm in thickness with the Zimmer® electric dermatome (Zimmer® Ltd, Swindon, Wiltshire, UK) and put onto Whatman® filter paper. The prepared skin samples were cut into circles (approximately 15 mm in diameter) using a punch and hammer, wrapped in aluminium foil, sealed in a plastic Ziploc® bag, and stored in a freezer at -20°C until utilised (within 24 h). Prior to the skin permeation studies, the circles were left at room temperature to thaw. The skin circles were then mounted onto the vertical Franz diffusion cells.

2.2.4.2. Membrane release and skin diffusion studies

Twelve amber vertical Franz diffusion cells were utilised during diffusion studies. Either skin or a polytetrafluoroethylene (PTFE) membrane was mounted between the two compartments of the diffusion apparatus; the skin with the stratum corneum facing upwards in the direction of the donor compartment. Membrane release studies were conducted to determine whether the API was released from the formulation. Skin diffusion studies, on the other hand, were conducted to determine where the API would be available for pharmaceutical activity, e.g. in the stratum corneum-epidermis, in the epidermis-dermis, and/or through the skin into the systemic circulation.

Each receptor compartment was filled with 2 ml PBS (pH 7.4) and the temperature of the cell system was maintained at 37°C. A magnetic stirring bar was placed in the receptor compartment to continuously mix the PBS (pH 7.4). The formulation (1 ml) was added to the donor compartment of the Franz cells to ensure skin saturation. Each donor compartment was closed and vacuum grease was applied to seal the Franz cells in order to avoid leakage; thereafter the two compartments were secured with a horse-shoe clamp.

At pre-determined time intervals the entire receptor volume was withdrawn and replaced with fresh PBS at pH 7.4 (37°C). The time intervals for the membrane release studies were hourly
up to 6 h; and for the skin diffusion studies every 2 h up to 12 h. This was to ensure that sink conditions existed throughout the experiment. Samples were directly assayed by means of HPLC to determine the API concentration in the receptor fluid.

2.2.4.3. Tape stripping

All the skin circles were removed after the diffusion studies. They were fixed onto filter paper and all excess formulation was dabbed from the skin with a clean paper towel. Fifteen tape strips were used to remove the stratum corneum-epidermis. After stripping the stratum corneum-epidermis off the diffusion area, the fifteen tape strips were placed in a polytop containing a 40% methanol in PBS solution at pH 7.4. The polytop was vigorously shaken to wet all the strips and left overnight (approximately 12 h) to be analysed by HPLC the following day.

The diffusion area of the remaining skin circles (epidermis-dermis) was cut out and also cut into pieces (to enlarge the surface area). These pieces were placed in a polytop containing a 40% methanol in PBS solution (pH 7.4). The polytop was vigorously shaken and stored overnight to be analysed by HPLC the following day.

2.2.4.4. Data analysis

The average cumulative amount per area (µg/cm²) of the permeated API was plotted against time. The steady-state slope of the average cumulative amount per area against time provided the flux (µg/cm².h) of the API, whereas the average cumulative API amount per area versus square root of time (h⁰.⁵) presented the lag time for each of the formulations. The percentage flurbiprofen yielded from the total amount of API applied in the formulations was plotted for the skin and membrane studies.

2.2.4.5. Statistical data analysis

Inferential statistics involved one-way analysis of variance (ANOVA) and also parametric assumption testing (omnibus tests for differences between group means) ²⁸.

Levene’s test was employed to determine whether the variances of the four formulation groups were equal. It was found that the deviation from normality and difference in variation were not severe, though one-way ANOVA, as well as more robust tests were performed to assess the differences. After performing the omnibus tests, post-hoc follow-up tests were conducted to determine which formulation’s average cumulative concentration varied from one other ²⁹.

Furthermore, since skin diffusion studies represented repeated measures taken over time, a mixed model analysis was fitted to the data to account for the dependence structure. The Type
III test for fixed effects was applied as an omnibus test to indicate statistical significant difference between the levels of factors time and treatment (formulation). Pairwise comparisons of estimated marginal means were employed, after a Bonferroni adjustment for multiple comparisons was applied, to determine which levels differed significantly.

3. RESULTS AND DISCUSSION

3.1. SOLUBILITY AND DISTRIBUTION COEFFICIENT DETERMINATION OF FLURBIPROFEN

The ideal solubility of an API to permeate through the skin is more than 1 mg/ml. The solubility determined was 0.0570 mg/ml in water (pH 4.75, 32°C) compared to 0.0300 mg/ml (25°C) found in literature and 0.0704 mg/ml in PBS (pH 4.69, 32°C). Both pH values are below 5, since flurbiprofen is an acidic compound and lowers the pH as it dissolves. These results indicated that flurbiprofen was only slightly soluble and, therefore, it could be expected that it would experience some difficulty in permeating through the skin.

Literature varies on the documented log P (partition coefficient) values for flurbiprofen. Log P is the ratio of concentrations of an API in a mixture of two immiscible phases (predominantly water and octanol) at equilibrium. The partition coefficient stipulates how freely a compound permeates from the formulation into the skin, whereas the diffusion coefficient measures how readily a compound diffuses through the skin. The diffusion coefficient is the partition coefficient of the ionised molecule at a certain pH. This describes an API’s lipophilic/hydrophilic character. Swart et al. provided an experimental value of octanol-buffer partition coefficient of 3.34 at pH 7.0, whereas other sources stipulated a value of 2.48 ± 1.06 in PBS at pH 7.4 and 37°C. Experimental results from this study delivered a distribution coefficient of 3.41 at 32°C in PBS at pH 5. Therefore, it was clear that flurbiprofen does not have an ideal distribution coefficient for skin permeation, and some difficulty will be encountered when delivering the API through the skin.

3.2. MEMBRANE RELEASE STUDIES

The following rank order was obtained for the formulations’ average cumulative amount API released per area: vitamin F >> control > EPO >> Pheroid™. The vitamin F formulation released the highest average percentage of flurbiprofen (3.04%) from the total amount applied (1 ml of a 1% flurbiprofen formulation) to the donor compartment of the Franz cells; and thus obtained the highest average cumulative amount released per area after 6 h (564.89 µg/cm²). In fact, the vitamin F formulation was the only formulation that delivered results which were significantly higher (p = 0.001) than that of the control formulation. On the other hand, the Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%).
which can be converted to the lowest average cumulative amount released per area (336.72 µg/cm²) after 6 h. This may be due to the fact that the Pheroid™ formulation contained an added lipophilic ingredient together with the vitamin F, which may cause the API not to be released as effectively since the API will remain in the more lipophilic phase i.e. in the cream.

### 3.3. SKIN DIFFUSION STUDIES

From the skin diffusion results it was clear that the EPO formulation depicted the highest average percentage flurbiprofen diffused (0.51%), even though it did not show the highest amount released per area from the formulation. However, the differences between the average cumulative flurbiprofen amount diffused per area for the EPO (94.14 µg/cm²) and the control formulation (91.53 µg/cm²) were not statistically significantly different (p = 1). The Games-Howell post hoc test also delivered p-values stating this fact (p = 0.989). The Pheroid™ formulation depicted the lowest average percentage flurbiprofen released (1.81%) and also illustrated the lowest average percentage flurbiprofen diffused (0.24%). Comparing the Pheroid™ formulation to the control, it was observed that the percentage flurbiprofen that diffused from the Pheroid™ formulation was approximately half of the percentage flurbiprofen that diffused from the control (0.49%). Average cumulative amounts per area of formulations containing flurbiprofen that diffused through the skin, could be placed in the following rank order: EPO > control > vitamin F >> Pheroid™ with statistical data from pairwise comparisons given in Table 1.

<table>
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<tr>
<th>Table 1:</th>
<th>P-values between different formulations for skin diffusion (12 h) after employing pairwise comparisons and applying Bonferroni adjustments for multiple comparisons. (Statistically significant differences indicated in bold).</th>
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A graphic representation of the average cumulative amount of flurbiprofen per area as a function of time of the different formulations can be seen in Figure 1.

<table>
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<tr>
<th>Figure 1:</th>
<th>The average cumulative amount per area of flurbiprofen that diffused through the skin as a function of time to illustrate the average flux for the different formulations used from 2-12 h (n ≥ 9).</th>
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Figure 1 clearly indicates that the EPO formulation attained the highest, and the Pheroid™ formulation the lowest average cumulative amount per area that diffused through the skin after 12 h. The average flux and average percentage diffused are indicated in Table 2.

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<th>Table 2:</th>
<th>The average flux and the average percentage flurbiprofen diffused for the four formulations after 12 h.</th>
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The control formulation had the highest average flux value (8.41 µg/cm².h) for flurbiprofen with the EPO formulation following closely (8.12 µg/cm².h); nevertheless these values did not differ statistically significantly. The Pheroid™ formulation depicted the lowest flux (4.08 µg/cm².h) for flurbiprofen at approximately half the rate of the control formulation. Chi et al. 15 found that although UFAs are potent penetration enhancers, they increase the lag time of a drug proportionally to the amount of double bonds in the UFA molecular structure (when comparing UFAs with the same carbon number) due to conformational kinks that hinder fatty acids to insert themselves into the lipid bilayers of the stratum corneum. However, when the fatty acids are finally packed within the skin, higher skin flux is attained due to widening of the channels in the stratum corneum 15. A study by Choi et al. 10 found that an increase in double bonds of the EFAs, i.e. from linoleic- (C18:2) to linolenic acid (C18:3), proposed a significant decrease in skin permeation of a lipophilic compound. Oleic acid and palmitoleic acid were more preferable. This might therefore explain why the EFA formulations showed no significant increase in API permeation compared to the control formulation.

Prottery 39 stated that the EFAs most abundantly found in the skin are linoleic acid and arachidonic acid. This might begin to explain the results found, since EPO contains 65.0-80.0% linoleic acid and vitamin F only 35.5%. Thus EPO will move more freely into, and through the skin since it encounters less obstruction from the skin. Linoleic acid is an essential element in the organisation and preservation of the skin barrier 40, 41. Vitamin F, and thus also Pheroid™, contain no arachidonic acid, which makes up 9% of the total epidermal fatty acids 17, 23. Since arachidonic acid is a major part of the skin’s fatty acids, these two penetration enhancers may encounter more obstruction from the skin compared to EPO, as they are less similar to the skin’s fatty acid composition. The Pheroid™ also contain an added lipophilic ingredient in relation to all the other penetration enhancers used, making the colloidal particles in the cream more lipophilic which is then more favourable for the lipophilic API to rather accumulate in the cream itself. This results in a lower amount of API that moved out of the cream, into, and through the skin 9.

Water is also considered a penetration enhancer since it hydrates the skin which leads to the swelling of corneocytes and possibly altering the intercellular lipid phase organisation. This, in turn, increases the skin surface temperature and increases blood flow 25. The skin consists of 15-20% water, but since each of the formulations contained the same amount of water, it would have had no influence in difference of permeated API concentration 9.

3.3.1. Tape stripping

The control formulation obtained the highest flurbiprofen concentrations in the skin. A higher concentration was observed in the epidermis-dermis (0.45 µg/ml), whereas a concentration of
0.26 µg/ml was present in the stratum corneum-epidermis. The EPO formulation presented the second highest concentration in the skin where a concentration of 0.37 µg/ml was obtained in the epidermis-dermis and 0.19 µg/ml in the stratum corneum-epidermis. A graphical box-plot representation of this data can be seen in Figure 2.

According to Figure 2, it was clear that higher concentrations of flurbiprofen were present in the dermis-epidermis. All the formulations used delivered a skew distribution of data with extreme outliers; as observed from the error bars. This fact can also be seen in the higher variation between the mean and median as indicated by the white and black lines, respectively. Furthermore, although the control formulation obtained the highest concentration flurbiprofen present in the skin, there was no statistical significant difference between the control, EPO and vitamin F formulations. The Pheroid™ formulation, however, were significantly lower than the other formulations.

Figure 2:  **Box-plot representing the concentration (n ≥ 9) flurbiprofen (µg/ml) present in the stratum corneum-epidermis and the epidermis-dermis for the different formulations after the skin diffusion studies. The mean and median are represented by the white dash and the black line dividing the box, respectively.**

The Games-Howell post hoc tests revealed a statistically significant difference between the flurbiprofen concentrations present in the stratum corneum-epidermis of the Pheroid™- and EPO formulations (p = 0.01); and the Pheroid™- and vitamin F formulation (p = 0.032). The difference between the control- and Pheroid™ formulations delivered a p-value of 0.062. A statistically significant difference was also found for the concentrations present in the epidermis-dermis for the Pheroid™- and control formulations (p < 0.05), Pheroid™- and EPO formulations (p < 0.05), and Pheroid™- and vitamin F formulations (p = 0.005). Furthermore, a statistically significant difference was obtained between the concentration present in the receptor phase of the Franz cells (diffused through the skin) and the concentration API present in the skin (stratum corneum-epidermis and epidermis-dermis) with p < 0.05.

The type of formulation furthermore has a marked influence on the delivery of an API. Barry noted that cream formulations (oil-water emulsion) may donate water to the SC and slightly increase the hydration; this may increase the effects on skin permeability to some extent since water opens up the compact structure of the horny layer. Tween 80, a component of the cream, is a surfactant which likewise enhances the penetration of compounds. Tween 80 may solubilise and extract lipid components after entering the intercellular regions of the stratum corneum; and/or interact and bind with keratin filaments that may result in a disruption within the corneocyte. The Pheroid™ formulation delivered a statistically significant lower flurbiprofen...
concentration into the skin and the receptor fluid as stipulated earlier. All the formulations contained 5% penetration enhancer, but the 5% Pheroid™ consisted of EFAs as well as a combination of other lipophilic compounds. The Pheroid™ formulation indicated the lowest flurbiprofen release during membrane release studies, which may collaborate the notion that the formulation containing the Pheroid™ are more lipophilic overall and the lipophilic marker would prefer remaining in the formulation rather than diffusing into or through the skin.

Overall, higher concentrations flurbiprofen were achieved in the epidermis-dermis compared to concentrations obtained in the stratum corneum. This may be explained by the mechanism of action of the fatty acids as penetration enhancers. Fatty acids increase the API diffusion coefficient by disrupting the intercellular lipid matrix of the stratum corneum. The fatty acid molecules enter the lipid bilayer where it forms microcavities and increases the free volume available for API diffusion. Even a slight increase in the fatty acid fraction in this free volume cavity can increase the diffusion coefficient significantly \(^3,^{10}\). Furthermore, this may, in part, explain why more API was present in the epidermis-dermis compared to the stratum corneum-epidermis. Choi et al.\(^{10}\) stated that the formation of a kink at the double bond add to the formation of free volumes for API to permeate into, kinks increase with the number of double bonds, thus an increase in double bonds should increase permeation. However, an increase in double bonds resulted in a decrease in skin permeation, which was thought to be due to steric hindrance limiting the fatty acid partitioning into the stratum corneum \(^{10}\). This may explain why fatty acids in this study did not significantly increase API permeation into, and through the skin compared to the control formulation.

For topical treatment, it was established that the control formulation presented a higher flurbiprofen concentration in the skin compared to the formulations containing a penetration enhancer. This may be explained by steric hindrance which was enhanced by kinks in the molecule conformation which in turn increased with an increase in the amount of double bonds as is the case with linolenic- and linoleic acid \(^{10}\).

4. CONCLUSIONS

Membrane release data revealed that the API was released from all of the formulations and was therefore available on the skin surface for possible diffusion through it. The vitamin F formulation depicted the highest flurbiprofen released and therefore this formulation provided the highest amount API available per area for diffusion compared to the other formulations. However, analysis of the skin diffusion data revealed the contrary. Results showed that the formulation containing EPO as penetration enhancer depicted the highest amount delivered per area transdermally. This may be due to the fact that skin already consists of approximately 15% linoleic acid and 30% oleic acid; thus less obstruction is encountered. Previous studies showed
that the enhancing effect of fatty acids follow the following rank order: linoleic acid > oleic acid > linolenic acid; though linolenic acid proved more of an irritant to the skin. EPO contains significantly high concentrations linoleic (65-80%) and oleic acid (6-11%), causing it to diffuse more readily through the skin. The stratum corneum-epidermis consists of approximately 41% ceramides of which linoleic acid is a precursor. This may also begin to explain why higher linoleic concentrations have higher enhancing qualities, since it is already part of the skin structure and may have encounter less obstruction from the skin barrier.

Interestingly, the control formulation containing no penetration enhancers provided the highest flux of flurbiprofen diffusing through the skin. However, again the EPO formulation illustrated a flux-value that did not significantly differ from the rate of flurbiprofen diffusing through the skin as provided by the control formulation (p > 0.05). The EPO formulation furthermore demonstrated the shortest lag time compared to the other formulations. A shorter lag time indicates that an API will more rapidly start to diffuse through the skin. Thus, the EPO formulation depicted a faster onset of diffusion of flurbiprofen through the skin, which may be explained by the fact that the fatty acids present in EPO is also part of the stratum corneum structure and, therefore, less obstruction is experienced by the stratum corneum permeability barrier.

The data obtained for the skin diffusion and tape stripping studies illustrated that a higher average concentration API diffused into the receptor fluid (representing systemic circulation) after 12 h (49.22 µg/ml) compared to the average concentration diffused into the skin (0.260 µg/ml for stratum corneum-epidermis; 0.448 µg/ml for epidermis-dermis) as can be seen from the control formulation. Moreover, the EPO succeeded in improving the penetration of the API through the skin, since the control formulation delivered a higher concentration API deposited into the skin and the EPO formulation a higher concentration API transported through the skin compared to the other formulations studied.

Tape stripping data suggested that this specific cream formulation containing fatty acids as penetration enhancers will be most effective if used for transdermal therapy rather than topical therapy, since very low API concentrations were present within the skin (stratum corneum-epidermis and epidermis-dermis), which might not have a significant therapeutic effect. Significantly higher concentrations were observed in the receptor phase which represented the concentration of flurbiprofen that diffused through the skin (transdermal). Previous studies have similarly indicated that UFAs such as linolenic- and linoleic acid are effective penetration enhancers for flurbiprofen.

Only a few products are available on the market that contains flurbiprofen as an API, one is TransAct® patches. These patches contain 40 mg flurbiprofen and a concentration of 0.0385
µg/ml can be found in the blood after a single application, observed at 13.8 h\textsuperscript{49}. Other products are Froben\textsuperscript® or Ansaid\textsuperscript® tablets which contain 100 mg or 50 mg flurbiprofen in white, sugar-coated tablets; Froben\textsuperscript® gel which contains 5% flurbiprofen in 20 g tubes \textsuperscript{50}; and Ocufen\textsuperscript® ophthalmic solution contains 0.03% flurbiprofen sodium \textsuperscript{51}. This indicates that there are not many transdermal products available on the market and future prospects may include a formulation for transdermal application, which will have enhanced delivery for better relief of pain and inflammation.

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Disclaimer

Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the NRF do not accept any liability in regard thereto.
References


Date of access: 18 October 2012.


Tables

Table 1: *P*-values between different formulations for skin diffusion (12 h) after employing pairwise comparisons and applying Bonferroni adjustments for multiple comparisons. (Statistically significant differences indicated in bold).

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<th></th>
<th>Pheroid</th>
<th>Vitamin F</th>
<th>EPO</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.001</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EPO</td>
<td>0.000001</td>
<td>0.824</td>
<td></td>
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<tr>
<td>Vitamin F</td>
<td>0.008</td>
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Table 2: The average flux and the average percentage flurbiprofen diffused for the four formulations after 12 h.

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<tr>
<th>Formulation</th>
<th>Average flux (µg/cm²h)</th>
<th>Average percentage diffused (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPO</td>
<td>8.12</td>
<td>0.506</td>
</tr>
<tr>
<td>Control</td>
<td>8.41</td>
<td>0.492</td>
</tr>
<tr>
<td>Vitamin F</td>
<td>7.44</td>
<td>0.440</td>
</tr>
<tr>
<td>Pheroid™</td>
<td>4.08</td>
<td>0.244</td>
</tr>
</tbody>
</table>
Figure 2: The average cumulative amount per area of flurbiprofen that diffused through the skin as a function of time to illustrate the average flux for the different formulations used from 2-12 h (n ≥ 9).
Figure 2: Box-plot representing the concentration \((n \geq 9)\) flurbiprofen \((\mu g/ml)\) present in the stratum corneum-epidermis and the epidermis-dermis for the different formulations after the skin diffusion studies. The mean and median are represented by the white dash and the black line dividing the box, respectively.